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National Cancer Institute

Frank J. Rauscher, Jr., *Director*

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Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems[1] (Third Edition)

R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott

Drug Evaluation Branch, Drug Research and Development
Division of Cancer Treatment, National Cancer Institute
National Institutes of Health
Bethesda, Maryland

[1] Received May 24, 1972.

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Foreword

"Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems," which described the antitumor screens of the National Cancer Institute (NCI), were first published in 1959 (1) and appeared in greater detail in 1962 (2). As a consequence of a growing body of data on drug effects in animal screens and in the clinic, as well as basic studies on the dynamics of tumor cell proliferation (3–8), the screening program has been continuously modified within the last decade with respect to the test systems used and the experimental conditions for their use (9–11). Although NCI contract laboratories have been regularly informed of protocol changes via amendments to protocols and specific instructions from the NCI staff, these changes have not been presented in comprehensive form to the scientific community in general. Thus, we felt that publication of this third edition of Protocols describing current NCI screening practices was appropriate.

Comparison of the second and third editions shows the following major similarities and differences.

1. The number of test systems for which specific protocols are presented has been reduced from 24 to six. In vivo systems presently included are L1210 and P388 mouse leukemias, mouse melanoma B16, mouse Lewis lung (LL) carcinoma, and rat Walker carcinosarcoma 256 (W256). The KB cell culture system continues to be used routinely for in vitro screening.

2. L1210 leukemia continues to be the system of choice for the initial screening of synthetic agents. The justification for the use of this system has been documented (3,4,9,11).

3. The routine use of P388 leukemia for the initial screening of crude natural products has been started. Materials demonstrating minimal but significant activity against P388 ($T/C \geq 125$) are then tested against L1210, both as crude extracts and as more purified fractions. Cell culture, where appropriate, continues to be used for major bioassay testing associated with fractionation of in vivo active natural products. The use of P388 and L1210 in sequence for natural product screening in vivo stemmed from unpublished data indicating that L1210 and P388 tended to respond to similar materials, with the exception of certain specific chemical classes, and that P388 was generally more sensitive than L1210 to materials showing activity against both. Thus, one might expect that a potential L1210-active component of a natural product might be present in the crude extract in insufficient amount for the activity to be manifested, but that concentration of activity using the more sensitive P388 system for bioassay might lead to the isolation of the L1210-active substance. In testing the validity of this hypothesis, approximately 12,000 crude plant extracts were screened against L1210 and P388. Those showing minimal confirmed activity against either system were retested against both during fractionation. Table 1 shows that the number of crude extracts with L1210 activity is insignificant. However, 3% were active against P388 as crudes, and of the P388 actives, 7.8% subsequently displayed L1210 activity during some stage of fractionation. The data indicate that the use of P388 and L1210 in that order for the screening of these 12,000 materials would have increased the number of L1210 actives from one to 28. This 0.23% yield of L1210 actives contrasted sharply to the $< 0.03\%$ yield experienced with the screening in L1210 of more than 49,000 crude plant extracts from the inception of the screening program through December 1971 (table 1). While P388 was instituted as a "pre-screen" for L1210 activity, the further development of a material on the basis of P388 activity alone was not excluded. At least two materials are in current development toward clinical trial on the basis of P388 activity (table 2), although the degree of activity required for acceptance of a material as a clinical candidate is greater for P388 than for L1210.

4. The protocol for W256 is presented because followup testing of materials originally uncovered in this screen is continuing although W256 is no longer used for presumptive screening.

5. The addition of slower-growing tumors to the screen is being implemented. Basic studies with respect to the kinetics of tumor cell growth have suggested that LL carcinoma (6)[1]

[1] Kline I, et al. Unpublished data.

TABLE 1.—Yield of L1210-confirmed active materials (T/C% $\geq$ 125) from crude plant extracts screened against both L1210 and P388

	No. active/ total No. tested	Percent
Crude plant extracts tested in L1210 and P388	(ca 12,000)	—
Crude plant extracts confirmed in L1210 and P388	1/12,000	< 0.01
Crude plant extracts confirmed in L1210 only	0/12,000	0
Crude plant extracts confirmed in P388 only*	361/12,000	3.0
P388 actives (crude) confirmed in L1210 during fractionation	28/361	7.8
Yield of L1210-active materials resulting from P388-L1210 dual screen	28/12,000	0.23
L1210 actives from screening of crude plant extracts against L1210 only over the years†	15/49,290	< 0.03

*Some had been active in W256, Sarcoma 180, or another system but not in L1210.

†Thirteen of 15 actives have shown P388 activity as fractions or crystalline materials; they were not tested in P388 as crudes. At the time this table was prepared (Jan 1972), two materials had not been tested in P388 as fractions or crystalline materials.

TABLE 2.—Number of materials passing Division of Cancer Treatment (formerly Chemotherapy Program) Linear Array Decision Point 2A* during calendar year 1971

Basis for passing Decision Point	No. of materials
Active in L1210	12
Active in P388	2
Active in W256	1
Active in reticulum cell sarcoma	1
Shows endocrine activity†	1
Shows specific biochemical blocking action	1
Shows immune enhancement	1

*This is the network planning and control system currently utilized in the management of the NCI's Division of Cancer Treatment (DCT); its principal feature is the delineation and positioning of component activities of the Program in logical order of occurrence (12). Activities are organized within flows or arrays depending on the degree to which they represent "in-line" activities required for the logical development of an agent toward clinical trial (Linear Array) or supplementary and complementary activities. Decision Point 2A of the Linear Array involves the initial decision of the DCT staff to proceed with the development of a new agent. The decision is based on the extent to which the agent meets current criteria for activity in a current NCI screening system, activity in other appropriate systems in lieu of sufficient activity in a current screen, and uniqueness of chemical structure if the latter is known. It should be noted that the decision to move a new agent into development to clinical trial is based on a degree of screening activity considerably greater than the minimal values (T/C% $\geq$ 125 for survival systems or $\leq$ 42 for tumor-weight inhibition) required for a determination of confirmed activity in primary screening as well as other pertinent factors (see Appendix I, Evaluation section).

†Recommended by the Endocrine Evaluation Branch, Division of Cancer Biology and Diagnosis, NCI.

and B16 melanoma (13) are appropriate tumors for uncovering drugs which may selectively destroy tumors with a relatively high percentage of nonproliferating but viable (and hence potentially proliferative) cells. While these tumors are not used routinely for screening all submitted materials, they are used extensively for additional testing of active drugs. The extent to which LL and B16 may be used for primary screening will depend on the results of current studies and on the amount of each synthetic material submitted to the Program. Protocols for primary screening in LL and B16 are included although specific methods for their use are still under investigation. Consequently, these protocols may undergo considerable change in the future.

6. With respect to screening methodology, the most important departures from the methods published in 1962 (2) are the elimination of "sequential" screening in favor of initial dose-response testing and the use of multiple schedules for the L1210 screen. The reasons for these changes relative to economy of drug usage and yield of active agents have been described (9,14).

7. In addition to the test systems for which specific detailed protocols are presented in this edition, other systems described in the 1962 protocols continue to be used for special purposes; for example, analog comparisons, studies of cross resistance, etc. Such studies are not routine and are requested individually by the NCI staff. Often they involve special experimental methods and are designed by the staff. Protocols for such ancillary and specialized systems are not presented in detail in the current edition although they are listed and

Table 3. Functions of Drug Research and Development (Contract and Intramural)

A. Office of the Associate Scientific Director, DR&D, DCT, NCI

1. Animal procurement, distribution, genetic control, and disease control.
2. Basic studies related to fundamental biologic and biochemical investigations of antitumor agents.

B. Drug Development Branch

1. Synthesis of new chemical agents.
2. Procurement and resynthesis of bulk chemicals and drugs for preclinical and clinical evaluation.
3. Development of new agents from fermentation, plant, and other natural products.
4. Development and production of clinical dosage forms for clinical trial.
5. Chemical quality control of bulk and formulated drugs.
6. Procurement of radiolabeled drugs for clinical, pharmacologic, and experimental studies.

C. Drug Evaluation Branch

1. Primary screening in vivo and in vitro and evaluation of new materials (Screening Section).
2. Biological quality control of bulk and formulated drugs (Screening Section).
3. Bioassays to monitor purification of fermentation, plant, and other natural products (Screening Section).
4. Studies of optimal drug routes and regimens, combination chemotherapy, and other means for improving drug effectiveness (Office of the Chief and Screening Section).
5. Development of new and improved laboratory methods for discovering, evaluating, and utilizing potential antitumor agents (Office of the Chief and Screening Section).
6. In vivo investigation of mechanisms of action and resistance to drugs of clinical interest (Screening Section and Biochemistry Section).
7. Studies leading to development of assays for immunotherapy and immunosuppression (Immunotherapy and Immune Response Modifiers Section in collaboration with Office of the Chief, DEB, and Associate Chief for Laboratory Research, DR&D).
8. Studies of AKR leukemia (Immunotherapy and Immune Response Modifiers Section and Screening Section).

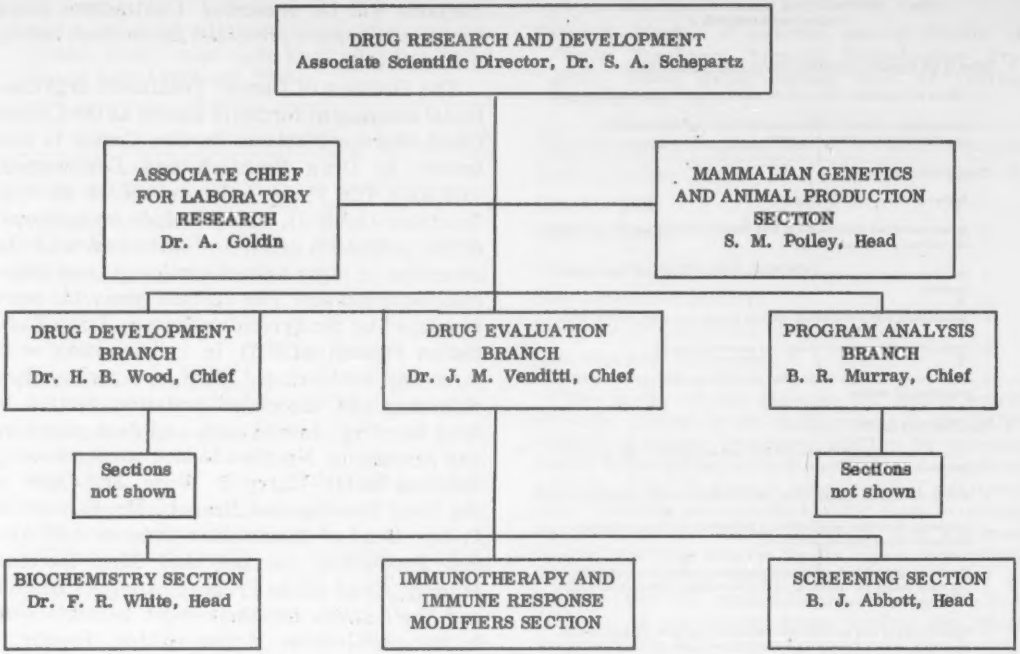
D. Program Analysis Branch

1. Development and operation of data processing systems for both biological and chemical information.
2. Search, retrieval, and dissemination of pertinent information from the world's literature on drugs and other aspects of cancer therapy.
3. Publication of the abstract journal Cancer Chemotherapy Abstracts.
4. Publication of the scientific journal Cancer Chemotherapy Reports.

described in summary form in Appendix II. Systems used for special aspects of drug evaluation and for which formal protocols have not been prepared include the spontaneous and transplanted forms of AKR leukemia and C3H mammary carcinoma. As these systems come into more regular, although limited, use, formal protocols describing appropriate experimental methods will be presented. Contractors using such systems have presented the current testing methods (15).

The Division of Cancer Treatment organizational component formerly known as the Cancer Chemotherapy National Service Center is now known as Drug Research and Development (DR&D) (fig 1) in order to reflect its true functions (table 3), which include management of the program's preclinical activities, with the exception of large animal toxicologic and pharmacologic studies. The current protocols were developed by the Screening Section, Drug Evaluation Branch (DEB) in collaboration with screening contractors.[2] They are pertinent to screening and associated activities related to drug handling, animal care, and data reporting and processing. Needless to say, we are heavily indebted to Dr. Harry B. Wood, Jr., Chief of the Drug Development Branch; Mr. Samuel M. Poiley, Head of Mammalian Genetics and Animal Production Section; and Mrs. Barbara Murray, Chief of the Program Analysis Branch and their staffs for their most valuable help in the preparation of this edition. Finally, I would like to extend my own personal thanks to Miss Betty J. Abbott (Head of the Screening Section, DEB) and to those members of her staff (Dr. R. I. Geran, Miss M. M. Macdonald, Mr. N. H. Greenberg, and Mrs. A. M. Schumacher) who worked so long and diligently in preparing this edition of the Protocols.

[2] Arthur D. Little, Inc. (P. Thayer, I. Wodinsky); Microbiological Associates, Inc. (I. Kline, R. Woodman); Southern Research Institute (F. Schabel, Jr., R. Laster, Jr., L. Wilkoff); Battelle Memorial Institute (R. Folk); Illinois Institute of Technology Research Institute (A. Shefner); Hazleton Laboratories (J. Gargus, L. Dudeck); WARF Institute, Inc. (B. Kline); Mason Research Institute (A. Bogden); University of Miami (M. Sigel, W. Lichter).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ASSOCIATE CHIEF FOR LABORATORY RESEARCH
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MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION
S. M. Polley, Head
DRUG DEVELOPMENT BRANCH
Dr. H. B. Wood, Chief
DRUG EVALUATION BRANCH
Dr. J. M. Venditti, Chief
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IMMUNOTHERAPY AND IMMUNE RESPONSE MODIFIERS SECTION
SCREENING SECTION
B. J. Abbott, Head

FIGURE 1.—Organizational components of Drug Research and Development.

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John M. Venditti
Chief, Drug Evaluation Branch
Drug Research and Development
Division of Cancer Treatment
National Cancer Institute

February 1972

L1210: Ascitic fluid implanted in BDF_1 or CDF_1 mice. Treatment begins 24 hours after implant. Results expressed as a percentage of control survival time. Under normal conditions, the inoculum site for primary screening is ip, the drug is administered ip, and the parameter is mean survival time (test system code, 3LE21; *see* Appendix I and Appendix II). For testing other than primary screening, the information in this Protocol may vary by instruction from DR&D.
Origin of tumor line: **induced in 1948 in spleen and lymph nodes of mice by painting skin with MCA.[1]**

ANIMALS (*see* Protocol 8)

Propagation: DBA/2 mice (or BDF_1 or CDF_1 for one generation if DBA/2 are not available).

Testing: BDF_1 (C57BL/6 × DBA/2) or CDF_1 (BALB/c × DBA/2) mice.

Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.

Sex: One sex used for all test and control animals in one experiment.

EXPERIMENT SIZE (*see* Protocol 9)

General Testing: Six animals per test group.

Control Groups: Number of animals varies according to number of test groups.

TUMOR TRANSFER (*see* Protocols 5 and 6)

Implant: Inject ip.

Size of Implant: 0.1 ml of diluted ascitic fluid containing 10^6 cells.

Time of Transfer for Propagation: Day 6 or 7.

Time of Transfer for Testing: Day 6 or 7.

TESTING SCHEDULE (*see* Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (*see* Protocol 7). Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.

Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (*see* Protocol 10). Treat as instructed.

Day 2: Recheck cultures. Discontinue testing if contaminated.

Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.

Day 20: If there are no survivors except those treated with positive control compound, evaluate experiment.

Day 30: Kill all survivors and evaluate experiment.

QUALITY CONTROL (*see* Protocol 7)

Acceptable control survival time is 8–11 days. Positive control compound is 5-fluorouracil (NSC-19893): single dose = 200 mg/kg/injection, intermittent dose = 60 mg/kg/injection, and chronic dose = 20 mg/kg/injection. T/C lower limit for positive control compound is $\geq$ 135%. Check control deaths, no takes, etc.

EVALUATION (*see* Protocol 11)

Compute mean animal weight on Days 1 and 5, and at the completion of testing compute T/C for all test groups with $>$ 65% survivors on Day 5. A T/C value $\leq$ 85% indicates a toxic test. An initial T/C $\geq$ 125% is considered necessary to demonstrate activity. A reproduced T/C $\geq$ 125% is considered worthy of further study. For confirmed activity a synthetic must have two multi-dose assays (each performed at a different laboratory) that produce a T/C $\geq$ 125%; a natural product must have two different samples that produce a T/C $\geq$ 125% in multi-dose assays.

REPORTING

On the final day of testing, prepare final control and test reports and send for key-punching.

[1] J Natl Cancer Inst 13(5):1328, 1953.

P388: Ascitic fluid implanted in BDF_1 or CDF_1 mice. Treatment begins 24 hours after implant. Results expressed as a percentage of control survival time. Under normal conditions, the inoculum site for primary screening is ip, the drug is administered ip daily for 9 days, and the parameter is median survival time (test system code, 3PS31; *see* Appendix I and Appendix II). For testing other than primary screening, the information in this Protocol may vary by instruction from DR&D.

Origin of tumor line: **induced in 1955 in a DBA/2 mouse by painting with MCA.[1]**

ANIMALS (*see* Protocol 8)

Propagation: DBA/2 mice (or BDF_1 or CDF_1 for one generation if DBA/2 are not available).

Testing: BDF_1 (C57BL/6 × DBA/2) or CDF_1 (BALB/c × DBA/2) mice.

Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.

Sex: One sex used for all test and control animals in one experiment.

EXPERIMENT SIZE (*see* Protocol 9)

General Testing: Six animals per test group.

Control Groups: Number of animals varies according to number of test groups.

TUMOR TRANSFER (*see* Protocols 5 and 6)

Implant: Inject ip.

Size of Implant: 0.1 ml diluted ascitic fluid containing 10^6 cells.

Time of Transfer for Propagation: Day 7.

Time of Transfer for Testing: Day 6 or 7.

TESTING SCHEDULE (*see* Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (*see* Protocol 7). Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.

Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (*see* Protocol 10). Treat as instructed.

Day 2: Recheck cultures. Discontinue testing if contaminated.

Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.

Day 20: If there are no survivors except those treated with positive control compound, evaluate experiment.

Day 30: Kill all survivors and evaluate experiment.

QUALITY CONTROL (*see* Protocol 7)

Acceptable median survival time is 9–14 days. Positive control compound is 5-fluorouracil (NSC-19893): single dose=200 mg/kg/injection, intermittent dose=60 mg/kg/injection, and chronic dose=20 mg/kg/injection. T/C lower limit for positive control compound is $\geq 135\%$.

Check control deaths, no takes, etc.

EVALUATION (*see* Protocol 11)

Compute mean animal weight on Days 1 and 5, and at the completion of testing compute T/C for all test groups with $>65\%$ survivors on Day 5. A T/C value $\leq 85\%$ indicates a toxic test. An initial T/C $\geq 125\%$ is considered necessary to demonstrate activity. A reproduced T/C $\geq 125\%$ is considered worthy of further study. For confirmed activity a synthetic must have two multi-dose assays (each performed at a different laboratory) that produce a T/C $\geq 125\%$; a natural product must have two different samples that produce a T/C $\geq 125\%$ in multi-dose assays.

REPORTING

On the final day of testing, prepare final control and test reports and send for key-punching.

[1] Scientific Proceedings, Pathologists and Bacteriologists 33(3):603, 1957.

Melanotic Melanoma B16

Protocol 1.300

B16: Tumor homogenate implanted ip or sc in BDF_1 mice. Treatment begins 24 hours after either ip or sc implant or is delayed until an sc tumor of specified size (usually approximately 400 mg) can be palpated. Results expressed as a percentage of control survival time. The drug is administered ip daily for 9 days and the parameter is median survival time (test system code, 3B131 or 3B132; *see* Appendix I and Appendix II); however, the procedures in this Protocol may vary by instruction from DR&D.

Origin of tumor line: **arose spontaneously in 1954 on the skin at the base of the ear in a C57BL/6 mouse.[1]**

ANIMALS (*see* Protocol 8)

Propagation: C57BL/6 mice.

Testing: BDF_1 (C57BL/6 × DBA/2) mice.

Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.

Sex: One sex used for all test and control animals in one experiment.

EXPERIMENT SIZE (*see* Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of animals varies according to number of test groups.

TUMOR TRANSFER (*see* Protocols 5 and 6)

Propagation: Implant fragment sc by trochar or 12-gauge needle or tumor homogenate (*see* below) every 10–14 days into axillary region with puncture in inguinal region.

Testing: Excise sc tumor on Day 10–14.

Homogenate: Mix 1 g of tumor with 10 ml of cold balanced salt solution and homogenize, and implant 0.5 ml of this tumor homogenate ip or sc.

Fragment: A 25-mg fragment may be implanted sc.

TESTING SCHEDULE (*see* Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (*see* Protocol 7). Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.

Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (*see* Protocol 10). Treat as instructed.

Day 2: Recheck cultures. Discontinue testing if contaminated.

Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.

Day 60: Kill all survivors and evaluate experiment.

QUALITY CONTROL (*see* Protocol 7)

Acceptable median control survival time is 14–22 days for ip tumor. Positive control compound is 5-fluorouracil (NSC-19893) (chronic dose = 20 mg/kg/injection) or cyclophosphamide (NSC-26271) (chronic dose = 50 mg/kg/injection). T/C lower limit for positive control compound is $\geq$135%. Check control deaths, no takes, etc.

EVALUATION (*see* Protocol 11)

Compute mean animal weight on Days 1 and 5, and at the completion of testing compute T/C for all test groups with >65% survivors on Day 5. A T/C value $\leq$85% indicates a toxic test. An initial T/C $\geq$125% is considered necessary to demonstrate activity. A reproduced T/C $\geq$125% is considered worthy of further study. For confirmed activity a synthetic must have two multi-dose assays (each performed at a different laboratory) that produce a T/C $\geq$125%; a natural product must have two different samples that produce a T/C $\geq$125% in multi-dose assays.

REPORTING

On the final day of testing, prepare final control and test reports and send for key-punching.

[1] Handbook on Genetically Standardized Jax Mice. Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, 1962. *See* also Ann NY Acad Sci, vol 100, Parts 1 and 2 (Conference on the Biology of Normal and Typical Pigment Cell Growth of 1961), 1963.

Lewis Lung Carcinoma — Protocol 1.400

LL: Tumor may be implanted sc as a 2–4-mm fragment, or im as a 2×10^6-cell inoculum. Treatment begins 24 hours after implant or is delayed until a tumor of specified size (usually approximately 400 mg) can be palpated. The drug is administered ip daily for 11 days, and the results are expressed as a percentage of the control. Procedures in this Protocol may vary by instruction from DR&D.

Origin of tumor line: **arose spontaneously in 1951 as carcinoma of the lung in a C57BL/6 mouse.[1]**

ANIMALS (*see* Protocol 8)

Propagation: C57BL/6 mice.

Testing: BDF_1 mice.

Weight: Within a 3-g weight range, with a minimum weight range of 18 g for males and 17 g for females.

Sex: One sex used for all test and control animals in one experiment.

EXPERIMENT SIZE (*see* Protocol 9)

General Testing: Six animals per test group for sc implant, or ten for im implant.

Control Groups: Number of animals varies according to number of test groups.

TUMOR TRANSFER (*see* Protocol 6)

Implant: Inject cells im in hind leg or implant fragment sc in axillary region with puncture in inguinal region.

Time of Transfer for Propagation: Days 12–14.

Time of Transfer for Testing: Days 12–14.

TESTING SCHEDULE (*see* Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (*see* Protocol 7). Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.

Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (*see* Protocol 10). Begin treatment as instructed.

Day 2: Recheck cultures. Discontinue testing if contaminated.

Day 5: Weigh animals and record (if applicable). Prepare fresh compound for subsequent testing.

Final Day: Kill all survivors and evaluate experiment.

QUALITY CONTROL (*see* Protocol 7)

Acceptable im tumor weight on Day 12 is 500–2500 mg. Acceptable im tumor median survival time is 18–28 days. Positive control compound is cyclophosphamide (NSC-26271): 20 mg/kg/injection, qd, Days 1–11. Check control deaths, no takes, etc.

EVALUATION (*see* Protocol 11)

Compute mean animal weight when appropriate, and at the completion of testing compute T/C for all nontoxic test groups. When the parameter is tumor weight, a reproducible $T/C \leq 42\%$ is considered necessary to demonstrate activity. When the parameter is survival time, a reproducible $T/C \geq 125\%$ is considered necessary to demonstrate activity. For confirmed activity a synthetic must have activity in two multi-dose assays (each performed at a different laboratory); a natural product must have activity in two different samples.

REPORTING

On the final day of testing, prepare final control and test reports and send for key-punching.

[1] Cancer Res 15:39, 1955.

Walker Carcinosarcoma 256 — Protocol 1.500

W256: Tumor may be implanted sc in the axillary region as a 2–6-mm fragment, im in the thigh as a 0.2-ml inoculum of tumor homogenate containing 10^6 viable cells, or ip as a 0.1-ml suspension containing 10^6 viable cells. Drug treatment is usually ip. Procedures in this Protocol may vary by instruction from DR&D.

Origin of tumor line: **arose spontaneously in 1928 in the region of the mammary gland of a pregnant albino rat.[1]**

ANIMALS (*see* Protocol 8)

Propagation: Randombred albino Sprague-Dawley rats.

Testing: Fischer 344 rats or randombred albino rats.

Weight Range: 50–70 g (maximum of 10-g weight range within each experiment).

Sex: One sex used for all test and control animals in one experiment.

EXPERIMENT SIZE (*see* Protocol 9)

General Testing: Six animals per test group.

Control Groups: Number of animals varies according to number of test groups.

TIME OF TUMOR TRANSFER (*see* Protocols 5 and 6)

Time of Transfer for Propagation: Day 7 for im or ip implant; Days 11–13 for sc implant.

Time of Transfer for Testing: Day 7 for im or ip implant; Days 11–13 for sc implant.

TUMOR TRANSFER (*see* Protocols 5 and 6)

Sc fragment implant is by trochar or 12-gauge needle into axillary region with puncture in inguinal area. Im implant is with 0.2 ml of tumor homogenate (containing 10^6 viable cells) into the thigh. Ip implant is with 0.1 ml of suspension (containing 10^6 viable cells) into the ip cavity.

TESTING SCHEDULE (*see* Protocols 3 and 4)

Prepare and administer drugs, weigh animals, and evaluate test on the days listed in the following table.

Test system	Prepare drug	Administer drug	Weigh animals	Evaluate
5WA16	2	3–6	3 and 7	7
5WA12	0	1–5	1 and 5	10–14
5WA31	0	1–9	1 and 5	30

[1] J Natl Cancer Inst 13(5):1356, 1953.

Day 0: Implant tumor. Run bacterial cultures (*see* Protocol 7). Determine solubilities. Thaw solutions. Run positive control in every odd-numbered experiment. Record survivors daily.

Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (*see* Protocol 10).

Day 2: Recheck cultures. Discontinue testing if contaminated.

Final Day: Kill all survivors and evaluate experiment.

QUALITY CONTROL (*see* Protocol 7)

Acceptable control weight or survival time for the following W256 test systems:

5WA16: 3–12 g.
5WA12: 3–12 g.
5WA31 or 5WA21: 5–9 days.

Check control deaths, no takes, etc.

Positive control compound is cyclophosphamide (NSC-26271): 2.5 mg/kg/injection.

EVALUATION (*see* Protocol 11)

Compute mean animal weight as appropriate, and at the completion of testing compute T/C for all nontoxic test groups. When the parameter is tumor weight, a reproducible T/C $\leq 42\%$ is considered necessary to demonstrate activity. When the parameter is survival time, a reproducible T/C $\geq 125\%$ is considered necessary to demonstrate activity. For confirmed activity a synthetic must have activity in two multi-dose assays (each performed at a different laboratory); a natural product must have activity with two different samples.

REPORTING

On the final day of testing, prepare final control and test reports and send for key-punching.

Cell Culture Screen, KB

Protocol 1.600

KB: KB cells are cultivated on Eagle's basal medium plus 10% serum. Stock cells are fed 24 hours before testing. Test material added on Day 0 or Day 1. Results are expressed as the dose that inhibits growth to 50% of control growth by 3 days after drug addition.
Origin of tumor line: **derived in 1954 from a human epidermoid carcinoma of the mouth.[1]**

EXPERIMENT SIZE

General Testing: Three to five dose levels per material. Two tubes per dose level.

Control Group: Number varies according to number of test groups (n), according to the formula: $2\sqrt{n}$. Determine baseline protein according to method of Oyama and Eagle.[2]

TEST SCHEDULE

Day 0: Dilute stock cells to 10–20 μg/ml (20,000–30,000 cells/ml) in complete media. Add cells to tubes and add test material simultaneously or on Day 1. Total volume is approximately 3–4 ml. Run positive control on odd-numbered control groups.

Day 1: If 24-hour culture is used, refeed and add test material. Determine protein values of baseline tubes.

Day 3: Conduct protein analysis of test, control, and at least three protein standard and media blank tubes.

Day 4: If 24-hour cultures are used, conduct protein analysis as prescribed for Day 3.

DOSAGE

Test synthetics and plant products by weight (W) at 100, 10, and 1 μg/ml.

Test crude fermentation products by dilution (D) at 1:10, 1:100, and 1:1000.

Test dried or crystalline fermentation products by weight at appropriate concentrations. Any material which does not reach an end-point at these levels is to be retested at lower concentrations. All additional tests are to be carried out at five dose levels at 0.3-log intervals.

QUALITY CONTROL

Control tubes must show growth of at least six times that of baseline tubes. Positive control, 6-mercaptopurine (NSC-755), limits ED50 between 0.05 and 0.5 μg/ml.

REPORTING

Mail test and control screening reports for data processing.

CRITERIA OF ACTIVITY

		First test	*Average (first and second tests)*
Sequential:	Synthetics	ED50 ≤6 μg/ml	ED50 ≤4 μg/ml
	Plant extracts	ED50 ≤30 μg/ml	ED50 ≤20 μg/ml
	Fermentation products	ED50 >1:100 dilution	ED50>1:100 dilution
Confirmation:	Synthetics	ED50 ≤4 μg/ml	
	Plant and animal extracts	ED50 ≤20 μg/ml	
	Fermentation products	ED50 >1:500 if known classes of cytotoxic agents are excluded	

[1] Eagle H. Proc Soc Exp Biol Med 89:362–364, 1955.
[2] Oyama VI, and Eagle H. Proc Soc Exp Biol Med 91:305–307, 1956.

2.000 The following instructions apply to the DR&D screening of materials as potential cancer chemotherapeutic agents. Deviations must be authorized in writing from DR&D by the appropriate staff member.

2.100 **SOURCES OF ANIMALS, TUMORS, AND CELL LINES**

All animals, tumors, and cell lines are to be obtained only from sources approved by DR&D. A list of accredited animal suppliers can be obtained from the Head, Mammalian Genetics and Animal Production Section, DR&D. Tumors and cell lines or information regarding them can be obtained from the Head, Screening Section, DEB, DR&D.

2.200 **TEST MATERIALS**

All materials tested under the auspices of DR&D must be identified by an NSC number and must be accompanied by a Materials Shipping List (Form NIH 1156) when received for testing by a screening contractor.

2.201 **Source of Test Materials:** Materials may be sent to the screening laboratory from DR&D, or directly from the supplier by written authorization of the Chief, DDB, DR&D.

2.202 **Assignment of NSC Numbers:** NSC numbers for synthetics are assigned by the DDB, DR&D. Natural products (B prefix numbers) are numbered by DR&D either by the assignment of a block of NSC numbers to a supplier or by the assignment of numbers to individual materials. For fermentation products the supplier assigns NSC numbers to specific samples in numerical order from within his block. The instructions for the assignment of suffix codes are contained in Instruction 31, which is available upon request from the Chief, PRA, DR&D.

2.203 **Quantity of Test Materials:** The screening laboratory should weigh, measure, or estimate the quantity of materials immediately upon receipt. The quantity scheduled to be received should be verified and any discrepancy should be recorded on the Materials Shipping List.

2.204 **Handling and Storage of Test Materials:**

Materials known to be hazardous will be so indicated on the Materials Shipping List, but since any test material may be irritating, toxic, potentially carcinogenic, or otherwise hazardous, and these potential hazards may be unknown to DDB, DR&D, the contractor will be responsible for establishing reasonable laboratory procedures and controls to protect the safety of personnel and facilities. EMPLOYEES SHOULD BE INSTRUCTED TO FOLLOW PROCEDURES CONSISTENT WITH ACCEPTABLE LABORATORY TECHNIQUES DESIGNED TO GUARD AGAINST PERSONAL INJURY. When possible, unstable materials will be so designated by DDB, DR&D or the supplier, but if a material not so designated indicates evidence of instability (eg, color change), it should be treated as unstable and refrigerated or frozen. Such materials must be put into a form suitable for administration within 30 minutes before injection. Small quantities of material can be weighed and stored in suitable vessels, each containing enough material for one injection period. The appropriate quantity of diluent can then be added immediately before injection. After preparation for injection, all materials should be kept refrigerated in amber bottles (or frozen if appropriate).

2.205 **Disposition of Materials:** All samples of test materials submitted for testing remain the property of the Government. Except as generally instructed regarding transshipments, all test materials, upon completion of scheduled testing, will be returned to DR&D or other designated locations, destroyed, or otherwise disposed of in accordance with in-

structions from DR&D. However, if a test material is particularly expensive or scarce, DR&D may request the screener to freeze or store the remaining solution or suspension for additional testing later or for return to DR&D.

2.300 TEST SYSTEM ASSIGNMENT

Unless specified otherwise, all materials should be tested as directed in Protocols 12 and 13. If any in vivo test systems other than L1210 or P388 are to be scheduled, multi-dose assays rather than single-dose assays should be scheduled in these systems. Any additional testing will be conducted upon specific request of the DEB, DR&D. The screening laboratory should verify and/or enter on the Materials Shipping List the test systems for which each material is being scheduled in your laboratory; and having verified the shipping list, this list should be mailed to the data processing contractor and to DR&D's specified distributing agent.

2.400 TRANSSHIPMENT OF MATERIALS

All materials shipped from one screener to another must be accompanied by a Materials Shipping List which indicates type of material, quantity being sent, and reason for transshipment.

2.401 Transshipment for Cell Culture Testing (*see* Protocol 12.300)

(*a*) A 30-mg sample of each new synthetic material is to be transshipped promptly for cell culture (if quantity permits without interference with in vivo testing) when received by the in vivo screener.

(*b*) A 50-mg sample of each new plant and animal material (identified with an NSC number in the B600,000, B700,000, or B800,000 series, with no suffix) is to be transshipped for cell culture testing (if quantity permits) when received by the in vivo screener.

(*c*) A 50-mg sample of the confirmation sample (eg, B002) of each new active plant or animal material should be transshipped for cell culture testing.

2.402 Transshipment for In Vivo Testing: Synthetics which demonstrate activity in an in vivo test system should be rescheduled at the first laboratory and sent promptly to a second laboratory for confirmation of activity without individual instructions from DEB, DR&D. Compounds should be accompanied by a Materials Shipping List. If in insufficient quantity, report as "QNS" to Screening Section Project Officer, DEB, DR&D.

2.500 USE OF ANTIBIOTICS

The use of antibiotics and/or other therapeutic measures intended for the improvement of animal health and maintenance of tumor lines shall not be used without prior written approval by the appropriate Section of DR&D.

3.100 PREPARATION OF TEST MATERIALS FOR PRIMARY SCREENING

Routine Procedure: For primary screening, a suitable form for the parenteral administration of synthetics or natural products is a clear solution or a satisfactory suspension. The physical state should be indicated on Form NIH 1158 if it is not a solution.

To establish the suitable form for a test material for primary screening, use as small a sample of the material as is practical for the appropriate steps described below until a satisfactory form is found. If one step does not produce a suitable form, proceed to the next step. When a suitable form is found, the quantity of material needed for the testing is then to be prepared by that chosen procedure. Saline is to be 0.85% NaCl in distilled water without a preservative and should be sterilized.

For chronic treatment in the primary screen, test materials should be prepared on Day 0 and Day 5 and kept refrigerated, unless directed otherwise.

To prepare a material for a multi-dose assay in the primary screen, the serial dilution procedure should be used, ie, a sample of the highest concentration to be tested should be prepared in a suitable form in a quantity that will be sufficient to conduct testing at all dose levels (with appropriate serial dilution). Except for the undiluted 2-ml injection used for natural products, or in special instances, the volume of injection for all primary screening should be uniform for all animals in the experiment (ie, in the control group and in all test groups in that experiment).

Step 1: Use as small a sample as is practical. Attempt to obtain a satisfactory form in sterile saline (0.85% NaCl in distilled water without preservative) for administration of the highest concentration to be tested. A satisfactory form for primary screening of synthetics or natural products is a clear solution or a suitable suspension. If a suitable form is not obtained, proceed to the next step.

Step 2: Grind another small sample of the material in a porcelain mortar with a pestle (or in an electrically powered or handoperated tissue grinder with a form-fitting pestle and tube), and again attempt to obtain a suitable form by slowly adding sterile saline while grinding. If this does not produce a satisfactory form, proceed to the next step.

Step 3: Grind another sample of the material in a porcelain mortar with a pestle (or in an electrically powered or handoperated tissue grinder with a form-fitting pestle and tube). Slowly add the "suspending vehicle" authorized by the DEB, DR&D,[1] with constant agitation of the suspension.

If a suspension is used, the material to be injected should be drawn into the syringe separately from the suspension which is being agitated with a magnetic stirrer. Tween-80 (1 or 2 drops) may be used to stabilize an emulsion, but no other procedures should be used for the preparation of test materials for the primary screen without DR&D approval. If the use of heat $\geq$ 60 C, an organic solvent, surfactants other than Tween-80 (which may be used for an emulsion only), or a sonifier appears to be advisable, specific authorization should be obtained from DR&D and a description of the procedure should be noted on Form NIH 1157.

3.200 PREPARATION OF SPECIFIC TEST MATERIALS FOR PRIMARY SCREENING

Endocrines, alkylating agents, purines, and other classes of chemicals are to be prepared and tested as any other new test material with these exceptions:

Endocrines: Any compound with an "E" suffix on the NSC number is to be administered sc unless otherwise directed.

[1] Currently, hydroxypropyl cellulose (Klucel) is being used.

Alkylating Agents: Alkylating agents are to be prepared fresh daily and injected within 15 minutes of preparation. When this is impractical (ie, for every-3-hour injections), multiple vials or aliquots may be prepared and stored frozen.

Frozen Natural Products: Frozen natural products are to be prepared as follows:

(1) Thaw in late afternoon of day preceding first treatment of animals. Leave material at room temperature until slushy, and then return it to refrigerator. If urgency requires a more rapid procedure, the sample may be thawed in water bath at 37 C for less than 1 hour, with special permission of DR&D.

(2) If material must be formulated more than twice, it is suggested that it be subdivided at the time the first test is started, labeled, and refrozen at –15 C for use as needed.

(3) Dissolve material in or dilute it with sterile physiologic saline.

(4) Once diluted, temperatures should be raised and lowered as rapidly as possible in this procedure, and the original sample should be returned to the freezer as soon as feasible.

(5) Store material in freezer at –15 C or less.

Natural Products: Natural products received as alcohol extracts should be prepared as follows:

(1) While grinding a small sample with a pestle, slowly add small quantity of sterile saline. If not satisfactory, proceed to step 2.

(2) To another small sample, slowly add 95% ethanol (do not exceed 50 μl/mouse/injection for chronic treatment). If satisfactory, slowly add sterile saline while stirring. If not satisfactory, proceed to step 3.

(3) Suspend material in the suspending vehicle authorized by DR&D.

Special Instructions: Materials such as fractions of natural products received with special instructions for testing should be prepared according to those special instructions.

3.300 ADMINISTRATION OF TEST MATERIALS FOR PRIMARY SCREENING

Synthetic compounds, antibiotic filtrates, and other natural products are injected ip once daily unless a different route or frequency of administration is requested by the supplier or DR&D. Where possible, a disposable 25-gauge needle, 5/8 inch or less in length, should be used.

3.301 Compounds bearing an NSC number with a letter "E" suffix are to be injected sc. Olive oil, sesame oil, or peanut oil, if designated by special instruction or by the Project Officer as the vehicle of use, are to be administered sc at a maximum volume of 0.2 ml/injection. When oral administration is requested by DEB, DR&D, the drug is to be administered by intubation. If the animals are to be fasted, fasting is to be from 5 PM the evening before until 1 hour after drug administration. Fasting is to be omitted if the frequency of treatment would prolong fasting to the point of excessive animal weight loss.

3.302 Animals should be weighed on the days specified in the protocol for that test system. For the primary screen, the dosage for each test group should be based on the original average animal weight for that test group.

3.303 **Quantity of Diluent:** The dose must be contained in a quantity of diluent which the host animal can tolerate well. This volume should not exceed approximately 5% of the body weight except for fermentation products. For primary screening the volume of diluent should be uniform for all animals in an experiment (ie, in the control group and in all test groups in that experiment). In general, these quantities are:

	Range of diluent (ml)	
	Mice	Rats
Ip administration		
Fermentation products	0.5–2.0	1.0–3.0
Other natural products	0.2–1.0	1.0–3.0
Other materials	0.2–1.0	1.0–3.0
Oral administration	0.2–1.0	1.0–5.0
Sc administration	0.2–1.0	1.0–5.0

When feasible, the lowest volume in the range should be used. Exceptions: (*a*) In no case should CMC, if used by special permission, be administered in an amount exceeding 0.4 ml for mice or 1 ml for rats and hamsters. (*b*) If more than 1 ml must be administered sc to mice, the dose should be divided among several sites, each site limited to 1 ml.

3.304 **Control Animals:** Control animals should be treated with the most stringent vehicle used for dissolving or suspending any test compounds in that experiment. The volume should equal that used for the majority of the test groups except that CMC may not exceed 0.4 ml in mice or 1 ml in rats.

3.400 PREPARATION AND ADMINISTRATION OF ANY TEST MATERIAL FOR A SCHEDULE DEPENDENCY OR ROUTE STUDY

See Protocols 3.100 and 3.200 for preparation and administration of materials used for testing in the primary screen, and Protocol 9.200 for testing procedures for schedule dependency or route studies.

3.401 Each material for a schedule dependency and/or route study will be prepared and handled on an individual basis; therefore, specific instructions regarding each study will accompany every request sent by DR&D (testing laboratory is to contact DR&D if such instructions are not received.)

Schedule dependency studies are to be given first priority (*see* Protocol 9.300). In the event that a schedule dependency study cannot be started within 2 weeks of receipt of the drug, the DR&D requester should be promptly notified.

3.402 Specific instructions will be received with each drug; however, the following will serve as a *general* guide.

a. A test material for a schedule dependency or route study should be prepared fresh daily. Alkylating agents and other unstable materials should be injected within 15 minutes of preparation.

b. A test material for a schedule dependency or route study should be prepared in a preservative-free diluent. Generally, either sterile saline (0.85% NaCl in distilled water) or sterile distilled water is used. Every effort should be made to use a true solution in schedule dependency and route studies. A true solution (ie, reduction of solute to molecular size) results in a clear solution which is not cloudy and has no particles. In the event that a true solution does not result from the instructions received, the screening laboratory should request additional instructions from the DEB requester.

c. Animals are to be weighed as specified in the Protocol for that test system and, in addition, on each day of injection and during the 60-day observation period where such data would appear to be of value. The volume of the injection for each animal should be based on the body weight of that animal in order to insure the proper dose level.

d. A multi-dose assay should be prepared by serial dilution of the highest dose level.

e. To minimize trauma and lessen the chances of death from injection, the needle gauge and length should be kept to a minimum size: for example, a 25-gauge ⅝ inch needle is recommended for solutions. Sterile disposable syringes and needles should be used if feasible.

f. When oral administration is requested, the drug is to be administered by intubation. If the animals are to be fasted, the fasting is to be

from 5 PM the evening before until 1 hour after drug administration. Fasting should be omitted if the frequency of treatment would prolong fasting to the point of excessive weight loss. No more than five iv injections should be given. The use of a "true" solution is of particular importance in iv treatment.

Drugs used in a schedule dependency study are considered to be active and therefore should not be reported to have produced "no takes" (*see* Protocol 11).

4.100 SELECTION OF INITIAL DOSES BASED ON WEIGHT

Whenever possible, test materials (including plant extracts) are to be submitted with their dry weight (or dry-weight equivalent) indicated and doses are to be selected and recorded on a dry-weight basis. Doses selected should be rounded off according to Protocol 4.404.

4.101 When an LD50 from a one-injection testing is supplied, start protocol testing at the LD50, with 400 mg/kg as the highest permissible dose (*see* Protocol 12 for subsequent dose levels).

4.102 When toxicity data are not available and compound is supplied in sufficient quantity (65 mg or more) start testing with 400 mg/kg as the highest dose and continue testing according to Protocol 12.

4.200 SELECTION OF INITIAL DOSES BASED ON DILUTION

4.201 Dilution is to be used as the unit for dosage when the terms g/kg, mg/kg, μg/kg, ng/kg, and IU/mouse are not applicable. When a material is given as a dilution the total volume of the test injection is usually 1 ml/animal. The dilution is expressed as the ratio "a/bcde" (without decimals), where "a" is the undiluted material and "bcde" is the final diluted material (of which a portion is injected). When feasible, the numerator "a" is one. The numerator of the expressed dilution must be constant in a multi-dose assay: it is usually one.

Quantity of undiluted material (ml)	Diluent added (ml)	Final volume injected (ml)	Dilution recorded
1	0	1	1/0001
0.5	0.5	1	1/0002
0.25	0.75	1	1/0004
0.125	0.825	1	1/0008

A double dose of undiluted material necessitates special handling. When a 2-ml injection of the undiluted material is included in a multi-dose assay, "2" should be the numerator for all dilutions in that assay. Thus, a dilution may be expressed in any fraction which does not involve decimals and which accurately indicates the concentration of the material.

Example of a multi-dose assay that includes a double dose of undiluted material:

Quantity of undiluted material (ml)	Diluent added (ml)	Final volume injected (ml)	Dilution recorded
2	0	2	2/0001
1	0	1	2/0002
0.5	0.5	1	2/0004
0.25	0.75	1	2/0008

4.300 SELECTION OF SUBSEQUENT DOSES BASED ON OBSERVED TOXICITY

4.301 **Definitions of Toxicity:**

a. In a tumor-inhibition study, a test is considered toxic if 34% of the animals have died by the day of sacrifice.

b. In a survival study, a test is considered toxic if any one of the following conditions is met:

1. 34% deaths by the significant day for that test system (Day 5 for 3LE21 with early drug treatment); or
2. T/C $\leq$85%; or
3. in L1210 and P388, with early drug treatment, a negative average animal weight change difference (test minus control) $\geq$4 g by the significant day for that test system.

c. Deaths in *a* and *b.1* above are considered to be due to acute toxicity; deaths in *b.2* and *b.3* are considered to be due to chronic toxicity.

4.302 After the occurrence of acute toxicity, doses should be reduced according to the following schedule:

Percent deaths	Fraction of dose
0–34	No change
35–75	0.50
76–100	0.25
100 (in 24 hrs)	0.20

4.303 After the occurrence of chronic toxicity, the dose is reduced by half.

4.304 If a single-dose assay test yields a toxic result, the subsequent test is scheduled with a lower dose level in accordance with the above table with the following two exceptions:

a. A toxic single-dose assay test against L1210 by one injection should be followed by a multi-dose assay by one injection at lowered doses.

b. A toxic single-dose assay test following a status code 11 or 13 should be repeated at the same dose level. If the second test is toxic, the dose is then lowered.

4.400 RECORDING OF DOSES

This section delineates the proper methods for recording doses on screening test record forms to minimize ambiguities and errors.

4.401 **Synthetics and Other Materials Administered on a Weight Basis:** Synthetics and other materials administered on a weight basis should be reported in milligrams per kilogram per injection.

4.401a No two identical treatments (dose, route, interval, etc), can be run for any one sample of a material within a single control. Normally, doses should be recorded in milligrams per kilogram per injection, but the symbols "mg/kg/injection" are not to be recorded on Form NIH 1158. The screening test record form (NIH 1158) has six columns for recording the dose, with the decimal point between the fourth and fifth columns. Thus, the dose may range from 0.01 to 9999 mg/kg/injection.

4.401b Symbols for dose weight amounts.—If the dose is <0.01 mg/kg/injection, it should be recorded in micrograms per kilogram per injection and capital "M" should be recorded in the far right-hand column of the dose field. If the dose is >9999 mg/kg/injection, it should be recorded as grams per kilogram per injection and a capital "G" should be recorded in the right-hand column of the dose field (eg, 9999 mg/kg/injection is to be recorded as 9.9 G).

4.401c *Uniformity within a multi-dose assay.*—All dose levels in a multi-dose assay, where everything is the same except the doses, should be expressed in the same units. Thus, if any one dose must be expressed in micrograms, grams, nanograms (N), or international units (U), all doses in that series should also be expressed in these same units. The decimal point must remain in the same position. Since the unit designation M, G, N, or U fills one column, only five columns remain for the dose—four integers and one decimal place. Thus, a multi-dose assay expressed in micrograms can range from 0.1 M (0.0001 mg/kg/injection) to 9999 M (9.99 mg/kg/injection). A multi-dose assay expressed in grams can range from 0.1 G (100 mg/kg/injection) to 9999 G (9,999,000 mg/kg/injection).

4.402 **Crude Antibiotic Filtrate and Other Materials Administered as Dilutions:** Dilutions should be recorded according to Protocol 4.200.

4.402a No two identical treatments (dose, route, interval, etc) can be run for any one sample within a single control.

4.402b *Standard doses greater than 1 ml.*—In both rats and mice the dose administered should be based on 1 ml/animal. Thus, if 2 ml of undiluted material is the standard amount used, it should be recorded as 2/0001. If the 2/0001 dose is toxic and requires the dose in a subsequent experiment to be cut in half, then 1 ml should be administered and recorded as 1/0001 (*see* Protocol 4.200).

4.402c *Symbols for dilutions.*—Dilutions more dilute than 1/9999 must be expressed by special symbols indicating dilution factors. A dilution factor of 100 should be expressed with a capital C in the far right-hand column. This means that

the denominator is to be multiplied by 100. A dilution factor of 1000 is expressed by a capital K in the far right-hand column. The use of such symbols leaves only three columns for expressing the final volume. Thus, the dilutions that use a factor of 100 may range from 1/001 C (1/100) to 1/999 C (1/99,900). With the factor 1000, dilutions may range from 1/001 K (1/1000) to 1/999 K (1/999,000).

4.402d *Multi-dose assays.*—Within each multi-dose assay all dilutions must be expressed in the same unit. Thus, if any dilution is expressed by the factors C or K, all dilutions in that group must also use C or K.

4.402e *Decimal points.*—For dilutions, the decimal point is always implied to be to the right of the far right-hand column. If a C, a K, or another symbol is entered in the right-hand column, the decimal point is implied to be immediately to the left of that symbol.

4.403 **Dose Numbers:** Record a dose-sequence number for each dose in a multi-dose assay. The largest dose or highest concentration should be dose 1. Examples: 500 mg/kg/injection = dose 1, 250 = dose 2, 125 = dose 3; or dilution 1/0001 = dose 1, 1/0002 = dose 2, 1/0004 = dose 3, 1/0008 = dose 4. Where a single dose is used, record a dose sequence number of "0."

4.404 **Rounding off Doses:** Only two spaces to the right of the decimal point are available for the machine-processing of a synthetic dose. Therefore, digits beyond this space are omitted for the purposes of machine-processing, the last machine-processed digit is not modified, and all actual digits are included in the calculation of lower serial dilutions. Example:

Actual series	Machine-processed series
25.0	0025.00
12.5	0012.50
6.25	0006.25
3.125	0003.12
1.5625	0001.56
0.78125	0000.78

5.100 GENERAL INSTRUCTIONS

All screening laboratories should obtain tumors for use in DR&D screening programs from sources designated by the DEB, DR&D. The coding for tumor sublines is described in Protocol 5.300.[1]

5.101 Tumors used in the primary screen will be replaced simultaneously at all screening laboratories at designated intervals: each new supply of tumor tissue will have its line, color code, and generation designated.

5.102 No fewer than two sublines of each tumor will be maintained at each screening laboratory. In the event of contamination, undue delay in the receipt of replacement lines, or other problems regarding tumor sublines, screener should notify his project officer and the tumor maintenance center responsible for supplying his tumor lines. He will be furnished a fresh supply of the same tumor.

5.103 After the first successful use of a new supply of tumor sublines from the maintenance center, old sublines will be destroyed.

5.104 Screeners should maintain a record of the tumor transfer generation of all tumor lines. In addition, screeners will record the genealogy of all of their tumor lines in the appropriate columns in the Screening Control Record, as indicated in Protocol 5.300.

5.200 TUMOR LINES AND HOST ANIMALS

The table in Protocol 5.400 lists the animal species and strain recommended for the maintenance of a tumor-carrier line and the suitable animal for conduct of the test.

[1] The coding for tumors is described in Appendix I.

5.201 Tumor material for implantation into test groups shall be propagated in the proper host animal according to specified transplantation techniques (*see* Protocol 6).

5.202 Material to be implanted into test groups may be propagated for one generation in the hybrid strains used in the conduct of the test.

5.300 DEFINITIONS

Tumor Subline: the 5-digit numeric code, such as 25R05, designating the line, color code, and generation of the tumor sample distributed by the DEB tumor source.

Line: the parent tumor line acquired from an approved source and designated by that source with a 2-digit numeric code.

Color Code: a portion of the tumor line maintained separately by serial transplantation and designated by color codes B (for blue), R (for red), etc. The purpose of color codes is to identify tumors available at specific intervals. Contract laboratories may establish additional color lines if needed by tumor dilution. This color designation is recorded to the right of the code for tumor line.

Generation: the sequential number assigned to the tumor line or subline after it has been transplanted into a new host (eg, if the L1210 tumor 25R04 [generation 4] has been transplanted into a new host, it is designated 25R05 [generation 5]).

Example: An L1210 control record (Form NIH 1157) showing the subline symbols 25R05 would be defined as L1210 line 25, subline R (red), in the fifth transplant generation.

5.400 TABLE OF TUMORS, HOST STRAINS, AND TRANSFER TIMES

Tumor	Propagate in strain—	Transfer time for propagation (Day)	Test in strain—	Transfer time for test (Day)
L1210 lymphoid leukemia	DBA/2	6 or 7	BDF_1 or CDF_1	6 or 7
P388 lymphocytic leukemia	DBA/2	6 or 7	BDF_1 or CDF_1	6 or 7
B16 melanotic melanoma	C57BL/6	10–12	BDF_1	10–12
Lewis lung carcinoma	C57BL/6	14	BDF_1	12–14
Walker 256 carcinosarcoma	Fischer or RAR	11–13	Fischer or RAR	11–13

5.500 ASCITES TUMORS

Prepare a smear of ascitic fluid, stain with Wright's or other suitable stain, and examine for typical tumor cells at each transfer. If less than 95% of cells are tumor cells, discontinue use of the line and inform project officer at DEB, DR&D.

6.100 GENERAL INSTRUCTIONS

Transplant tumors under aseptic conditions (under a glass hood or in a closed cubicle). Kill animals in a humane manner. Immerse each animal totally in 0.1% Zephiran (benzalkonium chloride) or equivalent before the tumor or ascitic fluid is removed from the donor. Use separate sterile instruments for surgical removal of each solid tumor and a separate sterile syringe for aspirating each ascites tumor.

6.101 In general, transplant tumors for maintenance of carrier lines into the site used for conduct of the test. Sc tumors are implanted in the axillary region with puncture in the inguinal region; im tumors are implanted in the thigh muscle; ascites tumors are implanted ip. Antibiotic treatment of animals used to maintain tumor lines is not permitted.

6.102 Use a single transfer generation of one carrier subline for any one experiment. No antibiotic treatment of test or control animals is permitted.

6.200 TUMOR FRAGMENT PREPARATION AND IMPLANTATION

Use donor tumors on the day of growth designated in the individual test protocol and in Protocol 5.400. Excise tumors aseptically and debride of necrotic material.

6.201 **Fragment Size:** As designated in individual test protocol.

6.202 **Holding:** Use sterile petri dish with small amount of buffered physiologic saline (no antibiotic), or equivalent, on ice.

6.203 **Implantation:** Use 12- or 13-gauge sterile trocar loaded with single fragment, either aspirated or handled by sterile forceps. Use a freshly sterilized trocar for each ten tumor fragments. No more than 30 minutes should elapse from the time tumor is removed from donor animals until it is transplanted into test animals. Implant all fragments from one tumor before excising and preparing the next tumor.

6.204 **Bacterial Cultures:** Use first two and last two tumor fragments from each donor tumor (*see* Protocol 7.100).

6.205 **Handling Animals:** Use separately identified holding cages for each set of animals receiving implants from one tumor. Hold 24 hours. Read cultures. Proceed as in Protocol 7.100.

6.300 TUMOR BREI PREPARATION AND IMPLANTATION

Use donor tumors on the day of growth designated in the individual test protocol. Excise tumor aseptically and debride of necrotic material. Mince with sterile scissors or tissue grinder and sterile screen.

6.301 **Dilution:** Add *sterile* saline (0.85% NaCl in distilled water without antibiotic or preservative) in the proportion specified in appropriate test protocol.

6.302 **Holding:** Use sterile glass container in ice bath. No more than 30 minutes should elapse from the time tumor is removed from donor animals until brei is implanted in test animals.

6.303 **Implantation:** Use 0.2 ml/animal unless otherwise stated in test protocol. Use needle no larger than 19-gauge. Do not refill syringe.

6.304 **Bacterial Cultures:** All animals in an experiment should receive the same brei; this brei may be composed of pooled donor tissue. Add 0.4 ml of pooled brei (after dilution) to broth in four separate tubes for culturing.

6.305 **Handling Animals:** Use separate holding cage for each group of animals receiving implants from one brei. Hold 24 hours. Proceed as in Protocols 7.100 and 1.

6.400 ASCITES TUMOR PREPARATION AND IMPLANTATION

Use donor tumor on day of growth designated in Protocol 5.400 or in the individual protocol. Withdraw fluid aseptically, inserting needle through abdominal wall from which skin has been removed.

6.401 **Holding:** Use sterile glass container in ice bath. Pool samples if more than one donor is used. Hold small quantity in separate container with heparin for cell morphology study (*see* Protocol 6.402) and cell count (*see* Protocol 6.403).

6.402 **Cell Morphology Test:** Place one drop of fluid from syringe on glass slide, make smear, and let dry. Stain with Wright's stain. Make differential leukocyte count. If fluid contains at least 95% lymphoblasts or lymphocytes, it can be used. If less than 95% of cells are lymphoblasts or lymphocytes, discard the material. If pooled fluid is used, first 4 drops of fluid should be removed from pool for cell count and morphology study.

6.403 **Cell Count:** Place remaining fluid from syringe in small container and dilute 10- to 100-fold with saline. Make cell count as for white blood cells.

6.404 **Implantation:** Dilute sterile ascitic fluid held in the ice bath so that inoculum required in test protocol is contained in 0.1 ml. Diluent should be sterile physiologic saline or balanced fluids such as Hanks', Locke's, Gey's, Earle's, or Tyrode's. Inject ip with 23-gauge needle. Use sufficient number of sterile syringes and needles so that none will be refilled from pool of donor fluid. No more than 60 minutes should elapse from the time fluid is taken from donor and diluted fluid is implanted in test animals.

6.405 **Bacterial Cultures:** Add 0.4 ml of pooled fluid (after dilution) to broth.

6.406 **Handling Animals:** Animals may be randomized into test groups on the day tumor is implanted. Read cultures at 24 hours (*see* Protocols 7.100 and 1).

The purpose of tumor quality control is to assure that tumors are free of gross bacterial contamination, have growth characteristics consistent with the prototype, are compatible with the host, and have consistent responses to known chemotherapeutic agents.

7.100 CONTAMINATION

Test four separately cultured fragments of solid tumors or four separately cultured samples (0.4 ml) of diluted ascitic fluid or brei.

A. Culture each tumor used to maintain tumor line. Culture each tumor used as donor for test.

B. Use thioglycollate broth, four tubes per tumor. Incubate half of the tubes at room temperature and half at 37 C for 48 hours. Read at 24 and 48 hours.

7.101 If no contamination is noted or if growth appears in only one tube at the 24-hour reading randomize animals and proceed with test. If two or more tubes show contamination at 24 hours, discard animals receiving implants from that tumor and adjust screening-control record sheet accordingly.

7.102 If contamination appears at 48 hours in two or more tubes cultured from one tumor, discard the whole group and discontinue the test. Record "+" in specified area on control-record sheet and record appropriate control status code ("5"). Attempt to identify organism. Report findings and disposition of the tumor line to Screening Section, DEB, DR&D as soon as possible.

7.200 GROWTH POTENTIAL

7.201 **Solid Tumors:** Control animals receive the tumor at the same time as the test animals and receive the same volume of the most stringent vehicle given to any test animals in that experiment. Average tumor size in control groups on the day of evaluation should fall between minimum and maximum limits set forth in the individual test protocols. Record should be kept of the mean tumor size for each control group, with individual tumor sublines identified. If a tendency toward increased or decreased tumor growth rate is noted, contact Screening Section, DEB, DR&D.

7.202 **Ascitic Tumors:** Control animals receive the same number of tumor cells as the test animals and the most stringent vehicle given to any test animals in that experiment. Average survival time of controls receiving implants with specific numbers of cells should fall within limits stated in individual protocols. Each subline of each ascites tumor should be titrated at each fifth transfer of the subline (those with numbers ending in "5" and "0"), as follows:

a. For each inoculum level, a group of ten animals of the same strain used in test groups should be used.

b. Based on cell count of donor animal fluid where 10^n = size of test inoculum, use inoculum levels of 10^{n+1}, 10^n, 10^{n-1}, 10^{n-2}.

c. Record median or mean survival time of each group and day of death of each animal in the group. Report each titration to Screening Section, DEB, DR&D.

d. Record should be kept of the mean or median survival time for each control group, with individual tumor sublines identified. If a tendency toward increased or decreased survival time is noted, contact Screening Section, DEB, DR&D.

7.300 "NO-TAKES"

Failure of individual implants to become established and grow progressively in host animals in control groups is termed "no-takes." (Failure of tumor growth in treated groups may be considered "no-takes" under conditions outlined in Protocol 11.)

No. of original animals in control group	No. of "no-takes" that are excessive
≤ 14	≥ 2
15–24	≥ 3
25–34	≥ 4
35–44	≥ 5
45–54	≥ 6

If there are excessive "no-takes" in the control group, assign appropriate control status code and record on both the screening control and screening test records.

7.301 In tumor systems evaluated on the basis of tumor weight, control "no-takes" are defined as tumors weighing ≤39 mg in control mice or hamsters or tumors weighing ≤99 mg in control rats.

7.302 In tumor systems evaluated on the basis of survival time of animals, "no-takes" are defined as those animals in control groups that live beyond the observation period stated in the individual protocols and in Protocol 11.

7.303 Continued occurrence of control groups with "no-takes" should be considered evidence of a serious problem which might involve the tumor, host, technique, or other aspects of the test. Measures should be taken to solve the problem and Screening Section, DEB, DR&D should be kept informed.

7.400 HISTOCOMPATIBILITY

Failure of tumors to become established and grow progressively in tumor-line propagation or evidence of tumor regression should be reported to Screening Section, DEB, DR&D immediately. More than 20% "no-takes" or regressions in a group of control animals should be considered presumptive evidence of incompatibility.

7.500 EXCESSIVE DEATHS

A control group is considered to have an excessive number of deaths if, by the day considered significant for survivors for that test system (eg, Day 5 for 3LE21 or Day 7 for 5WA16), the number of deaths is as follows:

No. of original animals in control group	No. of deaths that are excessive
≤14	≥ 2
15–24	≥ 3
25–34	≥ 4
35–44	≥ 5
45–54	≥ 6

7.501 Continued occurrence of deaths by the significant day in control groups should be considered evidence of a serious problem which may involve the animals, tumors, experimental technique, or other aspects of the test. Screening Section, DEB, DR&D should be kept informed of the efforts being made to solve the problem.

7.600 POSITIVE CONTROL COMPOUNDS

The positive control compound designated in Protocol 1 is to be scheduled as a test material with every odd-numbered experimental group of each tumor system, except as stated in A and B below for resistant tumor test systems and schedule dependency studies. Alkylating agents should be prepared fresh daily and other compounds should be prepared fresh on Days 1 and 5 and the solutions kept refrigerated.

A. Test systems involving the use of a tumor specifically resistant to a compound will have a positive and a negative control compound run as a test material with each experimental group. The negative control compound is the compound to which the tumor is specifically resistant.

B. In a schedule dependency study only one positive control compound test is needed to monitor the response of the pooled tumor tissue. In the past, positive control compounds insoluble in water were prepared as solutions in other vehicles.[1] At the present

[1] For example, the old procedure for preparing NSC-740 was to dissolve NSC-740 in sodium bicarbonate (2% in distilled water without preservative) and add enough of this 2% sodium bicarbonate solution to reach the desired volume. When diluting further for lower dose levels dilute with this 2% sodium bicarbonate solution.

The old procedure for preparing NSC-749 was to weight 200 mg of NSC-749 and place in a 250-ml beaker.

time, however, all compounds, including the positive control compound and such compounds as NSC-740 and NSC-749, are to be prepared according to Protocol 3.

7.700 CONTROL STATUS CODES

Based on the criteria given previously, a control status code should be assigned to each control group, as follows:

1. Satisfactory control
2. Excessive deaths
3. Excessive "no-takes"
4. Mean tumor weight (or survival time) is outside limits
5. Other reasons (contamination, etc)
6. Excessive deaths and excessive "no-takes"
7. Excessive deaths, median or mean survival time, and mean tumor weight are outside limits
8. Excessive "no-takes" and mean tumor weight (or survival time) are outside limits
9. T/C of positive control is outside limits at standard dose in otherwise satisfactory control

A. Test of positive control compound at standard dose is toxic in otherwise satisfactory control.

7.701 Where control status codes other than "1" are assigned to a control, the screening laboratory should carefully review all tests carried out under that control. If any test results are questionable because of the unsatisfactory nature of the control and the test is to be repeated, it should be assigned test status code "33".

Add 140 ml of distilled water. Add 5 ml of 5N NaOH. Mix until completely dissolved. Back-titrate with 4N HCl to pH 9.3 (this will require about 5.6 ml of 4N HCl). pH paper may be used for adjusting pH. Transfer to a 200-ml volumetric flask and dilute to 200 ml. The daily dose of 50 mg/kg for a 20-g mouse is contained in 1 ml.

7.800 DETAILS OF PREVIOUS QUALITY CONTROL

Test system	Treatment day(s)	Acceptable mean (or median) control tumor weight or survival time	Previous positive control* (NSC No.)	Positive control dose (mg/kg/injection)	Positive control limit (T/C%)	Day of evaluation and minimum tumor weight limits for "no-takes"
3LE21	Day 1	8–11 days	26271	100	≥ 135	18-day survivors
3LE21	Qd, Days 1,5,9	8–11 days	749	80	≥ 135	18-day survivors
3LE21	Days 1–9	8–11 days	749	50	≥ 135	18-day survivors
3PS31	Days 1–9	9–14 days	60339 or 27640	16 10	≥ 135	18-day survivors
3B131	Days 1–9	14–22 days	762 or 26271	0.5 50	≥ 135	30-day survivors
3LL12	Days 1–11	500–2000 mg	26271	25	≤ 42	≤ 39 mg
3LL36	Days 1–11	18–28 days	26271	25	≥ 135	30-day survivors
5WA16	Days 3–6	3–12 g	26271	2.5	≤ 42	≤ 99 mg
5WA12	Days 1–5	3–12 g	9706	0.05	≤ 42	≤ 99 mg
5WA21	Days 1–9	5–9 days	26271	2.5	≥ 135	18-day survivors

*Current positive control compound is NSC-19893 for most test systems (*see* individual protocols). *See* List of Compounds for complete chemical names of NSC numbers.

8.100 **SUPPLIERS**

Normally, animals are supplied by Mammalian Genetics and Animal Production Section (MG&APS), DR&D. In some cases animals may be procured from commercial sources under instruction by this Section. Purchased animals shall be obtained from sources accredited by MG&APS, DR&D.

8.101 Accreditation is accorded to a producer of laboratory animals if he can demonstrate that his management methods meet or exceed the Institute of Laboratory Animal Resources (ILAR) Standards for the Breeding, Care, and Management of Laboratory Animals.

8.102 Lists of accredited producers are revised and distributed periodically by MG& APS, DR&D.

8.200 **CARE**

Maintain all animals and animal rooms in accordance with the Standards for the Care of Laboratory Animals of the ILAR and the appropriate MG&APS instructions.

8.201 It is recommended that the following animal room categories be provided: (*a*) isolation, (*b*) holding, (*c*) testing, and (*d*) tumor donor.

8.202 **Isolation Rooms:** When the animals have been transferred to cages from the shipping crates, fed, and watered, they are to be moved immediately into an isolation room which is isolated as much as possible from the balance of the screening facility. Animals in these rooms shall be serviced by personnel assigned only to this task. If this cannot be arranged, the isolated animals shall be serviced after all other animals have received care. Do not store isolated animals in holding rooms or testing rooms.

8.203 Each shipment from each source should be grouped separately through isolation. Do not pool two or more shipments from one source or from several sources. Maintain chronologic, sex, and source identifications on cage record cards. Do not pool animals from two or more cages if dead and/or moribund animals have been removed from these cages.

8.204 **Isolation Periods:** Rats should be isolated for a minimum of 24 hours. When possible, mice should be isolated for 1 week and then transferred to sanitized cages and moved to a holding room. Questionable or marginal animals are not to be held in a holding room, but should be isolated longer. When necessary and when travel stress has not been excessive, the isolation period for mice may be reduced, and the isolation period may be waived if the mice reach an acceptable weight within 48 hours after receipt. If more than 10% of the animals die during isolation, the balance may be discarded by the consignee, with the approval of the Head, MG&APS, DR&D.

8.205 **Holding Rooms:** These rooms shall be used to maintain animals that have been in isolation 1 week. The holding room should, where possible, be used for only one species. Animals that develop rapidly and exceed weight limits should be used either for expanding tumor lines or for developmental studies, ie, toxicity, etc. The holding period should be extended to 3 weeks for healthy animals that appear to gain weight slowly. If they are still questionable, request a decision on prolonged holding. Animals for testing shall be selected from holding rooms. Only randomizing and weighing shall be done in these rooms. Unsatisfactory animals in groups of 50 or more shall not be killed without prior consultation with the MG&APS, DR&D.

8.206 **Testing Rooms:** These rooms shall be used only for animals that are being used in a test. These rooms shall not be used for the storage of large quantities of food, bedding, caging equipment, laboratory equipment, etc. When possible, a testing room should house only one species. Two or more strains of a species are acceptable.

8.207 **Tumor Donor Rooms:** These rooms shall be used to maintain only those animals that are used as tumor donors. When possible, each room shall house only one species.

8.208 Each room in all categories shall contain a minimum of 1 day's supply of food in closed containers, shall be provided with a sink or other source of clean water, and shall have a complete set of tools needed for routine care of the animals. Tools or other utensils, food, bedding, etc, shall not be moved from one animal room to another. Employees should be encouraged to maintain a high degree of sanitation in their daily activities and to wear a clean laboratory coat or uniform each day. They should be alerted to the importance of personal hygiene.

8.209 Temporary receptacles, instruments, and utensils used during the handling of animals for one experiment or from one supplier's shipment shall be thoroughly sanitized, and, if possible, sterilized before being used for a different experiment. It is preferred that either of the following methods be used:

a. Use a completely different set of equipment for each experiment.

b. Use a clean set of instruments and a sanitized cage-divider for each experimental group. This divider can be of throwaway cardboard or plastic or of stainless steel, which can be reused after sterilization. The use of the divider for test animals eliminates the necessity of removing animals from the cage during injections, weighings, etc, and also limits the transmission of inapparent disease from cage to cage.

8.210 The consignee of animal shipments, as an agent of the Federal Government, is responsible for reporting more than 25 unsatisfactory animals on arrival. The consignee or his official representative shall therefore carefully inspect all animals immediately on arrival and record an excessive number of missing or dead animals and animals apparently in poor health. He shall also spot-weigh at least 10% of what appear to be the smallest animals in order to determine whether the supplier is complying with the minimum shipping weight. Unsatisfactory animals shall be reported on the appropriate forms to the Head, MG&APS, DR&D. Missing and dead animals shall also be reported to the office of the common carrier and the supplier. The joint inspection report shall be completed as soon as possible.

8.211 Animal rooms will be subjected to periodic inspections by DR&D personnel. Information on all aspects of animal care is available from the Head, MG&APS, DR&D.

8.212 **Cages:** Animal cages shall be made of corrosion-resistant materials.

8.213 Cages for isolation and holding purposes shall have solid sides and bottoms. The maximum number of animals per cage and the minimum floor space per animal shall be as follows:

Species	Animals per cage	Floor space per animal (square inches)
Mouse	25	8
Rat	10	30
Hamsters	12	12

8.214 **Cages Used for Testing:** Solid sides and bottoms are preferable. Suspended cages with open-mesh floors are acceptable. The maximum number of animals and minimum floor space per animal shall be as follows:

Species	Animals per cage	Floor space per animal (square inches)
Mouse	10	8
Rat	6	30
Hamsters	6	12

8.215 Each cage shall be provided with a food holder for pelleted rations. It shall be made of corrosion-resistant material. Food shall not be offered to animals on the floor of the cage.

8.216 Water shall be offered to animals in a closed system comprised of a bottle, stopper, and tube. Bottles shall be of

clear glass and shall be sanitized prior to refilling.

8.217 Tubes for water bottles shall be formed of glass or stainless steel. These tubes shall be sanitized when bottles are cleaned.

8.218 The use of a perforated bottle cap in lieu of the stopper system is acceptable provided that (*a*) the openings in the cap are large enough for adequate flow, and (*b*) the assembly is placed in a well deep enough to allow ready access to debilitated animals.

8.219 **Climate:** The climate within all animal rooms shall be maintained at uniform levels. Room temperature (in degrees Fahrenheit) for mice and rats should be 74 (±2 degrees). Relative humidity should be maintained between 45% and 55%. Although mechanical difficulties may arise from time to time, the relative humidity should not be allowed to drop below 40% nor exceed 60%.

8.300 SACRIFICE

All laboratory animals shall be killed in a rapid, humane manner that will avoid creating needless pain and suffering. The use of such methods as decapitation, anesthesia, air embolus, and CO_2 asphyxiation are acceptable. The method selected shall not in any way alter or damage the tumor associated with the study in progress, nor so change its biological characteristics as to interfere with the evaluation of the experiment. The number of animals placed in a container used for euthanasia shall be limited in accordance with the floor space of the container. Mass killing of animals, involving placing animals in an overcrowded container and bringing the animals in direct contact with an anesthetic such as liquid ether, dry ice, or chloroform, is not to be used.

8.400 DISEASES AND PARASITISM

Animals, excreta, and serum samples shall be selected in accordance with the MG&APS, DR&D instructions for this purpose, and shipped to the DR&D diagnostic laboratories designated, as scheduled by MG&APS, DR&D.

8.401 **Salmonella:** Send fecal samples to Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201, or to IIT Research Institute, 3441 South Federal Street, Chicago, Illinois 60616, as scheduled by DR&D. Animals or animal organs should be sent if requested.

8.402 **Ectromelia:** Send serum or plasma samples from animals, tumor samples, and animals to New Jersey College of Medicine and Dentistry, Jersey City, New Jersey. This laboratory also supplies vaccine for the prevention of infectious ectromelia. Ship tumor fragments in glycerol. Shipment of tumor samples is unscheduled and samples should be sent as required. Shipment of serum and plasma samples is scheduled by DR&D.

8.403 **Histopathologic Examination of Tissue:** (*a*) Please communicate with MG&APS, DR&D concerning shipping instructions. (*b*) It is often preferable to ship sick animals rather than tissue. (*c*) Ship tissue in glycerol or formalin.

8.404 **Antigenic Typing of Tumors—Histocompatibility Studies Involving Tumors and Hosts:** Ship suspect material in accordance with instructions supplied by the laboratory to Roswell Park Memorial Institute, Buffalo, New York. This is an unscheduled activity. Samples will be sent to other diagnostic laboratories as designated.

8.405 Supply all pertinent information with each sample or specimen shipped to the diagnostic laboratories.

8.406 Ectoparasites shall be controlled by means of authorized dusting powders and liquid sprays. Information on safe substances will be supplied by MG&APS, DR&D.

It is recommended that animals be sprayed with an authorized insecticide when they arrive at the facility if they are suspected of harboring ectoparasites. This can be done before they are

removed from the shipping crate. Insecticides shall be applied to interior building surfaces as a preventive measure for parasite control. Residual forms are preferred. Toxic compounds should be applied with paint brushes or low-pressure spraying devices. If the latter are used, it is strongly urged that animals be removed from the room prior to application. Materials shall not be used without prior MG&APS, DR&D approval. Every effort shall be exerted to prevent the escape of animals and/or the ingress of stray animals from outdoors. For the control of escapees, the establishment of bait stations is more effective than trapping. Information on the use of bait stations can be obtained from MG&APS, DR&D. *Do not* use Trichlofon or Vapona.

8.407 Animals that have been killed should be removed in disposable plastic bags. This method avoids the necessity of sanitizing reusable containers.

8.408 Antibiotics and/or other therapeutic measures intended for improvement of animal health shall not be used without prior written approval by MG&APS, DR&D.

8.500 GENERAL UTILIZATION

The consignee of animals supplied by the DR&D is urged to maintain close liaison with MG&APS, DR&D on quality of animals.

8.501 All requests for animals to be furnished by the DR&D shall be directed to the Head, MG&APS, DR&D.

8.600 WEIGHTS

Recorded animal weights should be accurate to the nearest 0.5 g. All animals in an experiment should be within the weight range specified for that test system: in general, for mice the weight range is 3 g and for rats the weight range is 10 g.

8.700 REPORTS

Consignees of animals should complete and forward Weekly Animal Report (Form NIH 5771).

Protocol 9 describes the testing procedures for primary screening and the general procedures for schedule dependency and route studies.

9.100 PRIMARY SCREENING

9.101 Number of Animals for Primary Screening and Fractionation Studies: When feasible, all of the animals for one experiment should be from one source.

Table 1: to be used when the test protocol specifies six animals per group.

	No. of animals in—	
No. of tests	Each treatment group	Control group
1–6	8	18
7–21	7	30
22–25	6	30
26–30	6	33

Table 2: to be used when the test protocol specifies ten animals per group.

	No. of animals in—	
No. of tests	Each treatment group	Control group
1–4	16	24
5–13	12	33
14–24	10	40
25	10	43

Table 3: to be used in emergencies when the use of fewer animals per test group in multi-dose assays has been authorized. If authorized in writing by Screening Section, DEB, DR&D, use the following number of animals in each test group:

a. Use four animals per test group in all test systems in which the primary screening protocol requires six animals (such as L1210 or P388).

b. Use six animals per test group in all test systems in which the primary screening protocol requires ten animals (such as B16).

c. The following table may be used to determine the number of control animals for table 3 experiments:

No. of test groups	No. of animals in control group
16–25	20
26–36	24
37–49	28
50–64	32
65–81	36

9.102 Number of Test Groups per Experiment:

a. No more than 25 tests should be carried out in one experiment with a single control group unless approved by Screening Section, DEB, DR&D. Test only one frequency and route of drug administration in one experiment when feasible.

b. When testing according to table 1 (*see* Protocol 9.101), the total number of tests under that control number shall not exceed 30. When testing according to table 2, the total number of tests under that control number shall not exceed 25.

c. Each dose level in a multi-dose assay shall be considered a separate test for purposes of selecting number of animals per test, number of animals needed for the control, and summary reporting of number of tests under a given control number.

9.103 Deaths should be recorded once daily, before drug injection.

9.200 SCHEDULE DEPENDENCY OR ROUTE STUDIES

Schedule dependency studies are to be planned and conducted in such a way that the data will permit a meaningful and valid comparsion of routes and regimens. Screening Section, DEB, DR&D should be advised promptly by letter of any unusual observations or problems noted during the course of the experiment that would contribute to the proper interpretation of the data. (Submit Form NIH 153 with the control pack.) All frequencies and routes of administration for the schedule dependency study of one compound should be scheduled simultaneously, using the same control number, the same strain, sex, and weight range of animals (within 5 g), and the same donor tumor tissue. Each schedule dependency study must include one positive control compound test group. Each different frequency or route

of drug administration should be considered a separate "treatment."

A dose-zero test is to be scheduled for each multi-dose assay; this group will receive vehicle only by the specified schedule of administration. The data for the dose-zero test should be processed so it will be included on the screening data summary for the particular drug being tested. The purpose of this test is to monitor the effect of the vehicle alone.

If the general health of the animals is questionable, contact DEB, DR&D requestor before scheduling the schedule dependency or route study. Animals are to be weighed as specified in the protocol for that test system and, in addition, on each day of injection and during the observation period where such data seem advisable. Doses (mg/kg/injection) should be adjusted to the most recent average animal weight.

Deaths are to be recorded at the same time daily, before drug injection. If it is observed that the animals die within 15 minutes of drug injection, deaths should be recorded as of the following day but the time of death should be noted under "Comments" on Form NIH 1158.

Animals should be kept and observed for a period of 60 days, at which time healthy survivors will be killed. Survivors showing signs of disease or toxicity should be held longer and DR&D should be notified by letter.

9.201 **Size of Control and Test Groups:** For schedule dependency studies other than survival systems, specific instructions will be sent by Screening Section, DEB, DR&D when the study is requested. For schedule dependency studies in a survival system, the number of animals used will be the following if the drug quantity permits:

	No. of animals with tumor	No. of animals without tumor (AA)
Animals for each dose level of drug	10	8
Animals for dose-zero test (vehicle for drug)	10	8
Control animals receiving no drug and no vehicle	60	24

If drug quantity does not permit use of the above number of animals, contact DEB, DR&D requestor.

The tumored control animals with no drug and no vehicle will serve as the control for all tumored test groups in the study and will be used as the "C" for the calculation of the T/C percentages for all treatments. Testing and reporting of mice without tumors (AA; test system code) should parallel those of mice with tumors.

9.202 Doses for schedule dependency or route studies (as with all other tests) are to be reported *per injection* (rather than per day) on Form NIH 1158, reports, charts, tables, and summaries. In survival systems median (rather than mean) should be used to evaluate a schedule dependency or route study.

9.300 TESTING PRIORITIES

A. As a general practice, antitumor testing of any material should be started within 2 months of its receipt and should then be processed through all of the testing for which it can be scheduled as soon as possible.

B. In scheduling experiments, materials will be selected for testing according to the following priorities:

1. *As soon as possible*—requests from DR&D marked "Priority No. 1," new analogs developed through DDB, DR&D contracts, D-K samples of natural products, and schedule dependency studies.
2. Confirmation of synthetics which have shown activity at a different laboratory.
3. Completion of testing of new

materials which have shown activity in this laboratory.

4. Labile materials (natural products and unstable synthetics).
5. Materials to be retested because of prior toxicity.
6. New materials designated on Materials Shipping List as of special interest to DDB, DR&D.
7. Other new materials should be handled in order of date of receipt.

Randomization procedures are based on the size of test groups for each specific test system, and are carried out after tumor implantation according to one of the two methods given below.

10.100 RANDOM-NUMBER SHEET METHOD

Screening Section, DEB, DR&D will distribute, on request, sheets of random numbers representing treatment groups already randomized for groups of four, six, and ten animals per group (sheets for groups of other sizes may also be obtained on request). The animals picked from a holding cage are assigned to treatment groups in the order of the numbers listed.

If the number of test (and control) groups in an experiment is less than the number on the randomization sheet, skip the higher numbers in making cage assignments.

Each list of random numbers should be used no more than ten times.

10.200 INDEX CARD METHOD

Prepare decks of index cards (3 × 5) equal to the average number of experiments run per week. New decks should be prepared every 3 months. Each deck should contain enough cards for the number of animals in an experiment (eg, 25 tests × six animals per test, plus 30 controls = 180). Decks may be re-used after a 3-year interval. On each card in a given deck write Treatment 1, Treatment 2, etc, so that each card represents an animal in a control or experimental treatment group. Make up control groups of the same number as test groups. Thus, for the example given, there would be five control groups of six animals each and 25 test groups of six animals each, for a total of 30 groups. From a table of random numbers, list a four-digit number on each card, following the pattern of entries in the table in a predesignated manner, eg, "read down," "read left to right," etc. Arrange cards in numerical order of random numbers. In the case of a tie, toss a coin. As each animal is picked from a holding cage assign it in order to the group listed on the card, ie, first animal goes to treatment group on first card, etc.

DR&D in vivo testing is evaluated by a variety of methods. The most commonly used methods are described below and include the recording of survival time, the measurement of tumor diameters, and the excision and weighing of local tumors. It should be noted that whenever a test is unsatisfactory due to faulty technical procedure, it should be assigned a test status code of "33" and repeated.

11.100 CALCULATION OF MEAN SURVIVAL TIME

Calculate the mean survival time according to the following formula:

$$\text{Mean survival time (days)} = \frac{\Sigma S + AS_{(A-1)} - (B+1)\,NT}{S_{(A-1)} - NT}$$

Example: for L1210, $\dfrac{\Sigma S + 6S_5 - 19\,NT}{S_5 - NT}$

Definitions:

Day A = Day on which deaths are no longer considered due to drug toxicity. *Example:* with treatment starting on Day 1 for survival systems (such as L1210, P388, B16, LL, and W256), Day A = Day 6.

Day B = Day beyond which control group survivors are considered "no-takes." *Example:* with treatment starting on Day 1 for survival systems (such as L1210, P388, and W256), Day B = Day 18. For B16, transplanted AKR, and LL survival systems, Day B is to be established.

ΣS = If there are "no-takes" in the treated group (as defined in protocol 11.103), ΣS is the sum from Day A through Day B. If there are no "no-takes" in the treated group, ΣS is the sum of daily survivors from Day A onward.

$S_{(A-1)}$ = Number of survivors at the end of Day (A–1). *Example:* for 3LE21, $S_{(A-1)}$ = number of survivors on Day 5.

NT = Number of "no-takes" according to the criteria given in Protocols 7.300 and 11.103.

11.101 The following is the method for calculating the standard deviation (SD) of the mean survival time of control animals, when requested by Project Officer or by Protocol for that test system. Use the factors in the table of Protocol 11.505. Do not compute SD for treated groups. To compute SD for controls, subtract the day registering the death of the first animal on or after Day A from the day registering the death of the last animal (through Day B) and multiply the difference by the factor shown for the number of animals used in the calculation. Animals dead before Day A or alive beyond Day B, which are not used in calculating the mean survival time, are also not used in calculating the SD.

Sample for L1210:

(A)

End of day	1	2	3	4	5	6	7	8	9	10	11
Survivors	30	30	30	29	29	29	27	16	4	2	1

(B)

End of day	12	13	14	15	16	17	18	19	20	21	22
Survivors	1	1	1	1	1	1	1	1	1	1	0

$(11–7) \times 0.247 = 0.988$

11.102 Compute T/C for all treated groups. T/C is the ratio (expressed as a percent) of the mean survival time of the treated group divided by the mean survival time of the control group.

11.103 Eliminate from calculations of the treated group animals surviving beyond Day B, according to the chart below:

No. of survivors in treated group beyond Day B	Percent of "no-takes" in control group	Conclusion
1	Any percent	Consider it a "no-take"
2	<10	Consider them drug inhibitions
	≥10	Consider them "no-takes"
≥3	<15	Consider them drug inhibitions
	≥15	Consider them "no-takes"

Positive control compounds should not be considered to have "no-takes" re-

gardless of the number of "no-takes" in the control group. Thus, all survivors on Day B are used in the calculation of T/C for the positive control. All known active drugs (such as in a schedule dependency study) should not be considered to have "no-takes" and surviving animals are to be used in the calculation of the T/C.

11.104 If an animal is accidentally killed during an experiment, strike through (but leave legible on the report) the number of original animals and survivors on days prior to the accidental death, and note above each the number of animals to be used in the calculation.

11.105 Evaluate and record survivors on day of evaluation as "cures" *or* "no-takes."

11.200 CALCULATION OF MEDIAN SURVIVAL TIME

A. Median Survival Time:
Median survival time is defined as the median day of death for a test or control group. If deaths are arranged in chronologic order of occurrence (assigning to survivors, on the final day of observation, a "day of death" equal to that day), the median day of death is a day selected so that one half of the animals died earlier and the other half died later or survived. If the total number of animals is odd, the median day of death is the day that the middle animal in the chronologic arrangement died. If the total number of animals is even, the median is the arithmetical mean of the two middle values. Median survival time is computed on the basis of the entire population and there are no deletions of early deaths or survivors, with the following exception:
If an animal is accidentally killed during an experiment, strike through (but leave legible on the report) the number of original animals and survivors on days prior to the accidental death, and note above each the number of animals to be used in the calculation.

B. Computation of Median Survival Time From Survivors:
If the total number of animals including survivors (N) is even, the median survival time (days) = $\frac{(X + Y)}{2}$, where X is the earliest day when the number of survivors is $\leq N/2$, and Y is the earliest day when the number of survivors is $\leq (N/2)-1$. If N is odd, the median survival time (days) is X.

C. Computation of Median Survival Time From Mortality Distribution:
If the total number of animals including survivors (N) is even, the median survival time (days) = $\frac{(X + Y)}{2}$, where X is the earliest day when the cumulative number of deaths is $\geq N/2$, and Y is the earliest day when the cumulative number of deaths is $\geq (N/2) + 1$. If N is odd, the median survival time (days) is X.

D. Examples of Distribution of Deaths and Survivors

Example*		Days after tumor inoculation																						No. of survivors on day of evaluation	Median survival time (days)
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20...	25...		
1	S	20	20	20	20	20	20	20	19	9	4	1		0											8
	D								1	10	5	3		1											
2	S	10	5	4	3	2	1	0																	1.5
	D		5	1	1	1	1	1																	
3	S	10	10	10	10	10	10	10	10	9	8	7	6	5	4	3	2	1	0						12.5
	D									1	1	1	1	1	1	1	1	1	1						
4	S	10	10	10	10	10	10	10	10	10	10	10	8	8	8	6	5	5	5	4	4	4	4	4	16.5
	D												2			2	1			1					
5	S	10	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	8	7	6	6	6	6	6	60†
	D								1									1	1	1					
6	S	10	10	10	10	10	10	10	10	9	8	5	5	5	5	5	5	5	5	5	5	5	5	5	35†
	D									1	1	3													
7	S	6	6	6	6	6	5	4	3	3	3	3	3	2	2	2	1	0							9.5
	D						1	1	1					1			1	1							
8	S	6	5	5	5	5	5	5	5	4	3	3	3	3	3	3	3	3	2	2	2	2	2	2	13
	D		1							1	1								1						
9	S	7	7	7	7	7	7	7	6	5	4	3	2	1	0										10
	D								1	1	1	1	1	1	1										
10	S	7	7	7	7	7	7	7	7	7	7	7	7	7	5	5	5	5	5	5	3	2	0		19
	D														2						2	1	2		
11	S	9	9	9	9	9	9	9	9	9	9	9	9	9	9	7	5	4	3	2	1	0			16
	D															2	2	1	1	1	1	1			
12	S	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	4	4	60†
	D																					2	1		

*S = survivors; D = deaths.

†In these examples, the day of evaluation is Day 60. Properly, each median marked † is greater than the indicated value. Inasmuch as the number of survivors on the day of evaluation is reported, such cases will be apparent.

E. Computation of Median Survival Time for Examples Shown in Table in Section D.

Example	N	N/2	Median survival time based on mortality distribution: $\frac{N}{2}+1$	X (Day)	Y (Day)	Median survival time (days)	Median survival time based on recorded survivors: $\frac{N}{2}-1$	X (Day)	Y (Day)	Median survival time (days)
1	20	10	11	8	8	(8+8)/2=8	9	8	8	(8+8)/2=8
2	10	5	6	1	2	(1+2)/2=1.5	4	1	2	(1+2)/2=1.5
3	10	5	6	12	13	(12+13)/2=12.5	4	12	13	(12+13)/2=12.5
4	10	5	6	15	18	(15+18)/2=16.5	4	15	18	(15+18)/2=16.5
5	10	5	6	60*	60*	(60+60)/2=60*	4	60*	60*	(60+60)/2=60*
6	10	5	6	10	60*	(10+60)/2=35*	4	10	60*	(10+60)/2=35*
7	6	3	4	7	12	(7+12)/2=9.5	2	7	12	(7+12)/2=9.5
8	6	3	4	9	17	(9+17)/2=13	2	9	17	(9+17)/2=13
9	7	3.5	—	10	—	10	—	10	—	10
10	7	3.5	—	19	—	19	—	19	—	19
11	9	4.5	—	16	—	16	—	16	—	16
12	7	3.5	—	60*	—	60*	—	60*	—	60*

*In these examples, the day of evaluation is Day 60. Properly, each median marked * is greater than the indicated value. Inasmuch as the number of survivors on the day of evaluation is reported, such cases will be apparent.

11.201 **Cures and "No-Takes":** It should be noted that "cures" and "no-takes" in systems evaluated by median survival time are based upon the day of evaluation (*see* Protocol 7.800). On the day of evaluation any survivor not considered a "no-take" as defined in Protocols 7.300 and 11.103 is to be recorded as a "cure." Survivors on day of evaluation are recorded as "cures" or "no-takes," but do not eliminate these from the calculation of the median survival time.

11.300 CALCULATION OF APPROXIMATE TUMOR WEIGHT FROM MEASUREMENT OF TUMOR DIAMETERS WITH VERNIER CALIPERS

The use of diameter measurements (with vernier calipers) for estimating drug effectiveness on local tumor size permits retention of the animals for lifespan observations.

11.301 When the tumor is implanted sc, tumor weight is estimated from tumor diameter measurements as follows. The resultant local tumor is considered to be a prolate ellipsoid with one long axis and two short axes. The two short axes are assumed to be equal. The longest diameter (length) and shortest diameter (width) are measured with vernier calipers. Assuming specific gravity to be approximately one, and π to be three, the mass (in mg) is calculated by multiplying the length of the tumor by the width squared and dividing the product by two. Thus,

$$\text{Tumor weight (mg)} = \frac{\text{length (mm)} \times (\text{width [mm]})^2}{2}$$

$$\text{or } \frac{L \times (W)^2}{2}$$

11.302 The reporting of tumor weights calculated in this way is acceptable inasmuch as the assumptions result in as much accuracy as the experimental method warrants.

11.303 When the tumor is implanted in a site other than subcutaneous, tumor weight is to be estimated according to instructions from DEB, DR&D.

11.400 CALCULATION OF TUMOR DIAMETERS

When requested by DR&D the effects of a drug on the local tumor diameter may be reported directly as tumor diameters without conversion to tumor weight.

11.401 To report tumor inhibition by comparing the tumor diameters of treated animals with the tumor diameters of control animals, the three diameters of a tumor are averaged (ie, the long axis and the two short axes).

11.402 A tumor diameter T/C of 75% or less indicates activity and a T/C of 75% is approximately equivalent to a tumor weight T/C of 42%.

11.500 CALCULATION OF MEAN TUMOR WEIGHT FROM WEIGHTS OF INDIVIDUAL EXCISED TUMORS

The mean tumor weight is defined as the sum of the weights of individual excised tumors divided by the number of tumors. This calculation is modified according to the rules listed below regarding "no-takes."

11.501 Small tumors weighing 39 mg or less in control mice and hamsters, or 99 mg or less in control rats, are regarded as "no-takes" and should be eliminated from the computations by omitting their weights from the sum of tumor weights and removing the number of "no-takes" from the total number of tumors. In treated groups, such tumors are defined as "no-takes" or as true drug inhibitions according to the following rules:

Percent of small tumors in treated group	Percent of "no-takes" in control group	Action to take
≤17	Any percent	Consider it a "no-take" and do not use it in calculations
18–39	<10	Consider them results of true drug inhibition and use them in calculations
	≥10	Consider them "no-takes" and do not use them in calculations

Percent of small tumors in treated group	Percent of "no-takes" in control group	Action to take
≥40	<15	Consider them results of true drug inhibition and use them in calculations
	≥15	Code all nontoxic tests "33"

Positive control compounds should *not* be considered to have "no-takes" regardless of the number of "no-takes" in the control group. Thus, the tumor weights of all surviving animals are used in the calculation of T/C for the positive control. Also, known active drugs should *not* be considered to have "no-takes," and all tumor weights are to be used in the calculation of the T/C.

11.502 Compute T/C for all treated groups having more than 65% survivors. The T/C is the ratio (expressed as a percent) of the mean tumor weight for treated animals divided by the mean tumor weight for control animals.

11.503 If an animal is accidentally killed or lost during an experiment, strike through (but leave legible on the report) the number of original animals and survivors on days before the day of the accidental death, and note above each the number of animals to be used in the calculations. Note the accidental death on the report. If additional (unaccounted for) animals are found in a cage, discard the experimental group, code the test "33," and note under "Comments."

11.504 Compute the SDs of the mean control tumor weight using the factors in the following table. Do not compute SDs for the treated groups.

11.505 Table for estimating standard deviation showing the estimating factor for SD given the range (difference between highest and lowest observation):

Size of sample	Factor (k)	Size of sample	Factor (k)	Size of sample	Factor (k)
2	0.886	21	0.364	41	0.230
3	0.591	22	0.260	42	0.229
4	0.486	23	0.256	43	0.228
5	0.430	24	0.254	44	0.227
6	0.395	25	0.252	45	0.226
7	0.370	26	0.250	46	0.226
8	0.351	27	0.248	47	0.225
9	0.337	28	0.247	48	0.225
10	0.325	29	0.246	49	0.224
11	0.315	30	0.244	50	0.224
12	0.307	31	0.243	51	0.223
13	0.300	32	0.241	52	0.223
14	0.294	33	0.240	53	0.222
15	0.288	34	0.239	54	0.221
16	0.283	35	0.238	55	0.220
17	0.279	36	0.236	56	0.219
18	0.275	37	0.235	57	0.219
19	0.271	38	0.233	58	0.218
20	0.268	39	0.232	59	0.217
		40	0.231	60	0.216

For example:

Given 7 observations	946*	814	550†
	832	702	
	861	680	

Estimate of SD: (946–550) × 0.370 = 146.5

*Highest.

†Lowest.

(This table is abstracted from Biometrik Tables for Statisticians [Pearson ES, and Hartley HG, eds]. Cambridge Press, vol 1, table 22, p 165.)

12.100 DEFINITIONS OF TERMS

A. *Test system* is designated by a five-digit code defining host group, tumor, parameter of evaluation, and site of tumor implantation. *Examples:* 3LE21, 3PS31.

B. *Test group* (*T*) is a group of animals that receive a specific dosage of a test material.

C. *Control group* (*C*) is a group of animals that receive only the most stringent vehicle given to any of the test groups in that experiment.

D. *T/C* is the mean or median survival time, diameter, or tumor weight of a test group divided by that of the control group expressed as a percentage.

E. *Control pack* consists of the data sheets (Forms NIH 1157 and 1158) for a single experiment which includes several test groups plus the control group.

F. *Control number* is the unique number within a given test system assigned to a single experiment. Numbers are assigned in sequence and are not reused. (If an experiment is unsatisfactory for any reason, the reason is noted, the control pack is submitted, and that control number is not reused.)

G. *Multi-dose assay* refers to the number of tests of one material in one experiment. It is used when the same material is tested at more than one dose level within the same experiment.

H. *Single-dose assay* refers to the number of tests of one test material in one experiment. It is used when the material is tested at only one dose level within one experiment.

I. *One-injection therapy* refers to the regimen of treatment with a drug, and is used when only one injection of the drug is administered during the entire time period of the experiment.

J. *Intermittent therapy* refers to the regimen of treatment with a drug, and is used when the interval between injections is greater than 1 day.

K. *Chronic therapy* refers to the regimen of treatment with a drug, and is used when the drug is given once a day for more than 1 day.

L. *Divided-dose therapy* refers to the regimen of treatment with a drug, and is used when the drug is given more than once a day.

M. Doses on a weight basis are expressed as *mg/kg/injection.*

N. *Activity* in a survival system is a reproducible $T/C \geq 125\%$.

O. In a survival system a multi-dose assay is considered to be "inactive and complete" when at least two nontoxic consecutive dose levels produce a $T/C < 125\%$ and at least one dose level is toxic (or is the highest dose authorized).

P. Toxicity is defined in Protocol 4.

12.200 TESTING IN TEST SYSTEMS OTHER THAN THOSE OF THE PRIMARY SCREEN

Testing in test systems other than those of the primary screen is conducted only upon special request from DEB, DR&D, and should be by a multi-dose assay unless otherwise specified.

12.300 FLOW OF COMPOUNDS THROUGH THE PRIMARY SCREEN

The retest procedures, including the test status codes, are described as follows:

12.301 In vivo testing takes priority over in vitro testing. When supply of drug is sufficient for both, all new synthetics, new plants, and new animal products are to be transshipped promptly for cell culture testing (*see* Protocol 2.400). Fermentation products are not routinely sent for cell culture testing at this time.

12.302 General Statements Regarding Primary In Vivo Screening of New Synthetics and Natural Products:

A. *Testing to be Scheduled Based on Supply Received*

1.—When the supply permits, each synthetic material is to be tested in the following order:

by intermittent administration against 3LE21 (q4d, Days 1, 5, 9);

by chronic administration against 3LE21 (qd, Days 1–9 at doses selected from previous test results); and,

against a second test system when designated by DR&D.

2.—When the supply permits, each natural product is to be tested in the following order:

by intermittent administration against 3PS31 (q4d, Days 1, 5, 9);

by chronic administration against 3PS31 (qd, Days 1–9 at doses selected from previous test results); and

by confirmed active 3PS31 regimen (if any) against 3LE21.

3.—If an LD50 for a single injection by a parenteral route is given, test by regimens in the following order as long as there is a supply of compound:

q4d, Days 1, 5, 9 at dose levels of x, $0.5x$, and $0.25x$

qd, Days 1–9 at dose levels of x, $0.5x$, and $0.25x$;

where x equals the single-injection parenteral LD50. However, do not exceed a dose of 400 mg/kg/injection or a 2/0001 dilution. If the LD50 is unknown and the supply is sufficient, test according to Protocol 12.303 for a synthetic and Protocol 12.304 for a natural product. Single-injection treatment is to be used only when the supply of material is limited.

4.—When the supply does not permit complete testing, test either a synthetic or a natural product as follows: if toxicity data are not supplied, test at doses of 400, 200, and 100 mg/kg/injection, by regimens determined by the screener based on the usable quantity of compound received. The suggested initial regimens listed below are intended only as guidelines for the screener to use under normal conditions to achieve the maximum utilization of each compound. The screener is to modify these assays where necessary because of animal weights, unique properties of a compound, or additional experience (eg, toxicity data from the first regimen will increase the amount of testing possible with a specified quantity of compound for further regimens and further test systems). If the quantity of drug is insufficient for a multi-dose assay, a single-dose assay is permissible.

If the screener receives (mg)	Initial regimens to be scheduled
≥ 1300	qd, Days 1,5,9 qd, Days 1–9
1100–1299	qd, Day 1 only qd, Days 1–9
975–1099	qd, Days 1–9
380–974	qd, Days 1,5,9
65–379	qd, Day 1 only
1–64	Transship for KB testing

B. Ideally, a single experiment contains only one regimen for all compounds.

C. Doses on a weight basis are expressed as mg/kg/injection (*see* Protocol 4).

D. After some data become available, the doses (or dilutions) for succeeding schedules should be adjusted accordingly, ie, the multi-dose assay for the qd, Days 1–9 schedule should have for its highest dose the highest nontoxic dose of the previous regimen (either intermittent or single).

E. *Positive Control Compound:*

Treatment regimen	NSC No.	Dose (mg/kg/injection)
One injection, Day 1	19893	200
Intermittent, qd, Days 1,5,9	19893	60
Chronic, qd, Days 1–9	19893	20

F. *Test Status Codes:* All doses of a multi-dose assay must be coded uniformly. The following test status codes are to be used when test status codes below 20 are not applicable.

No. of injections	No. of dose levels	Test status codes: Synthetics	Test status codes: Natural products
1, Day 1	multi	24	27
qd, Days 1,5,9	multi	25	26
qd, Days 1–9	multi	22	28
1, Day 1	single	23	21
qd, Days 1,5,9	single	23	21
qd, Days 1–9	single	23	21

A test which is unsatisfactory for any reason should be assigned a test status code of "33" and repeated promptly.

G. *Test Status Suffix Codes:* All doses of a multi-dose assay must be coded uniformly.

1.—Test data from all dose levels in the assay are toxic or inconclusive: no suffix.

2.—For synthetics: if one dose level in the assay is active, all doses are assigned the active suffix code "P." Otherwise, use "F."

3.—For natural products: see flow charts for Natural Products where the suffix codes are shown in parentheses.

12.303 In Vivo Testing of New Synthetics:

A. A new synthetic is first tested against 3LE21 by a three-dose-level assay q4d and then qd, Days 1–9; subsequently it is tested against a second test system when designated by DR&D.

B. Unless otherwise specified, all primary screening of a synthetic will be:

1.—by a three-dose-level assay at 400, 200, and 100 mg/kg/injection;

2.—at doses not exceeding 400 mg/kg/injection;

3.—by ip injection unless otherwise indicated (*see* Protocol 3.300);

4.—considered complete when the original supply is exhausted, except when:

a. an additional supply is needed for confirmation of activity, or

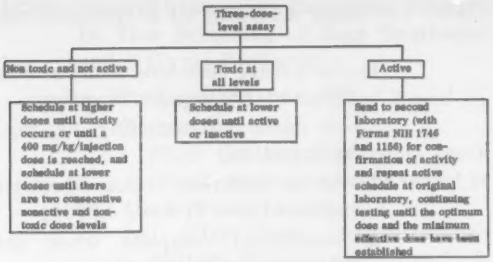
b. any regimen that has been started is incomplete, including one in which all dose levels tested were toxic, or

c. a second regimen has not been tested, and the first regimen produced activity;

5.—scheduled with priority given to confirmation testing of the active schedule. Confirmation testing at the second laboratory and repeat testing of the active schedule at the original laboratory should be conducted prior to the testing of additional schedules. Confirmation testing should be scheduled at doses of $1.5x$, x, and $0.66x$, where x equals the most effective dose from the active response data;

6.—continued until the supply is exhausted or until the specified schedules are fully tested. If, as a consequence of toxicity, schedules selected on the basis of quantity received are completed and the material is not yet exhausted, untested schedules should be run either by multi-dose assay or at a single-dose level depending on the amount remaining.

C. When supply is limited, *see* Protocol 12.302.A.4. When supply permits, the testing of synthetics for any one treatment regimen should proceed as follows:


Three-dose-level assay
Non toxic and not active
Toxic at all levels
Active
Schedule at higher doses until toxicity occurs or until a 400 mg/kg/injection dose is reached, and schedule at lower doses until there are two consecutive nonactive and non-toxic dose levels
Schedule at lower doses until active or inactive
Send to second laboratory (with Forms NIH 1746 and 1156) for confirmation of activity and repeat active schedule at original laboratory, continuing testing until the optimum dose and the minimum effective dose have been established

12.304 In Vivo Testing of New Natural Products:

A. A new natural product is first tested against 3PS31 by a three-dose-level assay q4d and then qd, Days 1–9. If it is confirmed active against 3PS31, the active 3PS31 regimen is then scheduled against 3LE21.

B. If toxicity data are not supplied, test at doses of 400, 200, and 100 mg/kg/injection, or at dilutions of 1/0001, 1/0002, and 1/0004, by regimens determined by the screener based on the usable quantity of compound received.

C. "Original" sample of a natural product refers to either the first sample received or the first sample used in a specific test system by a specific regimen. Thus, the original sample may carry sample suffix code B001–B005, etc.

D. Confirmation of a natural product requires activity with two *different* samples from the supplier. Only one regimen needs confirmation.

E. 1/0001 dilution = undiluted 1-ml injection, and 2/0001 dilution = undiluted 2-ml injection. In all other cases, dilutions are expressed as "a/bcde" where "a" is the volume of the undiluted material and "bcde" is the final volume to which the material is diluted (of which a portion is injected) (*see* Protocol 4). An injection of a natural product is never to exceed 2 ml of undiluted material.

F. Unless otherwise specified all primary screening of natural products will be:

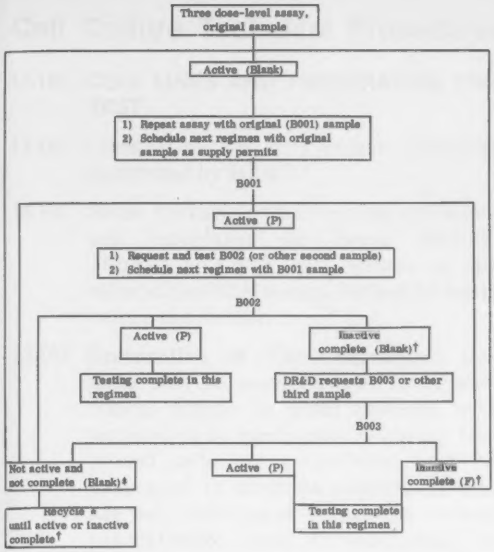
1.—at doses not exceeding 400 mg/kg/injection or 2/0001 dilution;

2.—by ip injection unless otherwise indicated;

3.—considered complete only when specified regimens have been tested.

4.—scheduled with priority given to followup testing of the active schedule. Both repeat testing of the active schedule with the original sample and confirmation testing with the additional sample should be given priority over the testing of additional schedules. However, testing of additional regimens should be continued while waiting for the second and/or third samples from the supplier. Repeat-testing of an active sample should be scheduled at doses of $1.5x$, x, $0.66x$, and $0.44x$ (where x equals the optimal dose and should not exceed 400 mg/kg), or $2y$, y, $0.5y$, and $0.25y$ (where y equals the optimal dose not greater than 1/0001). Dose levels for confirmation testing with additional sample should be at twofold intervals based on the toxicity (not the activity) of the active sample. However, if none of the doses in the followup testing of the active schedule are active, testing should be conducted at higher doses not to exceed upper limits of doses allowed.

G. For any one treatment regimen, the testing of natural products should proceed as follows:


Three dose-level assay, original sample
Active (Blank)
1) Repeat assay with original (B001) sample
2) Schedule next regimen with original sample as supply permits
B001
Active (P)
1) Request and test B002 (or other second sample)
2) Schedule next regimen with B001 sample
B002
Active (P)
Inactive complete (Blank)†
Testing complete in this regimen
DR&D requests B003 or other third sample
B003
Not active and not complete (Blank)‡
Active (P)
Inactive complete (F)†
Recycle * until active or inactive complete†
Testing complete in this regimen

( ) = last status suffix code.
* = Schedule higher until a 1/1000 dilution (or a 400 mg/kg/injection dose) or toxicity is reached; and/or schedule lower.
† = Inactive complete: there are two consecutive nontoxic nonactive levels.
‡ = This includes an assay that is toxic at all levels.

13.100 CELL LINES AND PREPARATION FOR TEST

13.101 Lines: Use KB cells unless otherwise instructed by DR&D.[1]

13.102 Stock Cultures: Cultivate as a stationary monolayer on Basal Medium (Eagle) plus 10% calf, human, or any other compatible serum. Refeed 24 hours before use on test.

13.103 Preparation of Cell Suspension: Cell sheet may be removed from glass with 0.25% trypsin in basal medium, with versene, or by mechanical scraping. Dispersed cells are centrifuged and resuspended in complete medium. If cells are not centrifuged, trypsin or versene concentration must be adequately reduced by dilution in complete medium. Determine protein count (after washing) on an aliquot according to the method of Oyama and Eagle.[2] Dilute stock cell suspension to 10–20 μg of cell protein/ml in complete medium. This will be about 20,000–30,000 cells/ml. Deliver uniform amount (3–4 ml) of cell suspension to culture tubes. Incubate at 37 C at 10° angle for 72 hours.

13.104 Preparation of Materials: Sterile techniques are to be used when setting up and dosing cultures. Minimal precautions necessitate a hood or "sterile" area with sterile instruments and glassware. Distilled water or saline are the solvents of choice to obtain a solution for testing. Other solvents may be used only after it has been determined that a material is not soluble in water or saline. With as small a sample as is practical attempt solubilization in saline. For materials not soluble in water or saline attempt to dissolve the material in alcohol; if still insoluble attempt other suitable solvents. Final concentration of the solvent may not exceed a predetermined noncytotoxic level. The final solution is made up to volume in complete media and when necessary the pH is adjusted to 6.5–7.5 with 0.25N HCl or NaOH. For completely insoluble materials, suspensions may be tested. Do not use suspending agents (eg, klucel, carboxymethylcellulose, or gum acacia) as they may mask true test results. Since suspensions may give false test results, these tests must be reported separately to DR&D as well as noted on the Screening Test Record (Form NIH 1376).

Materials designated as unstable are to be prepared immediately before testing. Repeat-testing, either to confirm "activity" or to establish an end-point, is to be with the original solvent. Synthetic materials and lyophilized natural products are not to be sterilized. However, all materials are to be handled as if sterile, and sterile techniques should be used throughout weighing and dosing procedures. Fermentation products are to be sterilized by filtration.

13.200 DOSE LEVELS

All synthetics and plant products are to be tested by weight (W), not dilution (D). First tests of synthetics and plant products are to be scheduled at 100, 10, and 1 μg/ml. Fermentation products, with a sample code of D through K may be tested by weight or dilution. All other fermentation products must be consistent (W or D) within the NSC core number. When tested by dilution, starting doses are 1:10, 1:100, and 1:1000.

13.300 EXPERIMENTAL DESIGN

Use a common set of control tubes to evaluate materials tested at one time (about 75). Use the number of baseline and control tubes according to the formula $2\sqrt{n}$, where n = number of materials being tested.

[1] Technical procedures for other cell lines, eg, Hep 2 and its resistant variants, are generally the same as those cited for KB.

[2] Oyama VI, and Eagle H. Measurement of cell growth in tissue culture with a phenol reagent. Proc Soc Exp Biol Med 91:305–307, 1956.

13.400 CALCULATIONS

Protein determinations are made for all test and control tubes, standards, and medium blanks according to the method of Oyama and Eagle.[3] Readings are made on a colorimeter at 660 mμ in the following order: protein standards and media blanks are read using a reagent blank, and then the optical density of each test and control tube is determined using the average media blank reading as zero.

13.401 The mean value of at least three standard tubes will be used for calculations of protein content.

13.402 The mean of the experimental tubes (T) for each dilution (dose) minus the mean of the baseline (C_0) divided by the mean of the control tubes (C) minus C_0 gives the growth ratio (Y) at each dose level.

$$\frac{T - (C_0)}{C - (C_0)} = Y \text{ or } \frac{\text{Treated}}{\text{Untreated control}}$$

13.403 The slope is the difference in response (Y) for a one-log difference in dose.

13.404 The ED50 is the calculated effective dose which inhibits growth to 50% of control growth.

13.500 QUALITY CONTROL

A. The final-day control tubes shall show growth of at least six times that of the baseline values.

B. The difference between optical density values of duplicate tubes at each dose level shall not be greater than 0.10.

C. The Y values shall not show a reversal of more than 0.10 between consecutive dose levels.

D. The positive control compound (6-mercaptopurine; NSC-755) is to be tested in every odd-numbered experiment. Quality control limits for 6-mercaptopurine are ED50 = 0.05–0.5 μg/ml.

[3] *See* footnote 2.

13.600 TEST EVALUATION

13.601 Initial Testing:

13.601a *Synthetics.*—Any material which has an ED50 $>$ 6 μg/ml in the initial test will be considered complete and no further testing will be scheduled unless specifically requested by DEB, DR&D. This is true whether or not an end-point has been obtained.

13.601b *Fermentation products.*—Any material for which the first test has an ED50 more concentrated than 1:100 (eg, 1:50 dilution) will be considered complete and no further testing will be scheduled unless specifically requested by DR&D. This is true whether or not an end-point has been obtained (*see* Protocol 12.301).

13.601c *Plant and animal products.*—Any material which has an ED50 $>$ 30 μg/ml in the initial test will be considered complete and no further testing will be scheduled unless specifically requested by DR&D.

13.602 Retesting Procedures to Establish End-Point:

13.602a *Synthetics and plant products.*—If the results of the first test show an ED50 $<$ 1.0 μg/ml, the screener will retest at lower concentrations, ie, 1, 0.1, 0.01, until the end-point is established.

13.602b *Fermentation products.*—If the results of the first test show the ED50 to be less concentrated (more dilute) than 1:1000, the screener will retest at higher dilutions, ie, 1:1000, 1:10,000, 1:100,000, until the end-point is established.

13.603 Retesting Active Materials:

13.603a *Synthetics.*—When the end-point is established as $\leq$ 6 μg/ml, the material is to be retested. If the average ED50 of the first and second tests is $\leq$ 4 μg/ml, the material is to be retested for confirmation.

13.603b *Plant products.*—Every plant product with an ED50 $\leq$ 30 μg/ml will be retested. If the average ED50 of the first

and second tests is $\leq$ 20 μg/ml, the material is to be confirmed with a different extract.

13.603c *Original fermentation products.*—If the ED50 is $>$ 1:1000, a refermentation sample from the supplier should be tested.

13.700 **NUMBER AND INTERVAL OF DOSE LEVELS**

The first test of any material for which the ED50 is unknown should include three dose levels at 1-log intervals. All additional testing, when a tentative end-point has been established, should be with five dose levels at twofold dilutions. This applies to tests at both the original screening laboratory and all other laboratories. Normally, the previously determined ED50 should be the third (middle) dose level of the five used.

13.800 **PRIORITIES**

Materials will be tested in the following order:

A. Specific test requests from DEB, DR&D indicating "priority," D-K fractions of natural products, and new synthetics developed through DR&D contracts.
B. Confirmation and necessary repeat testing to complete the testing of materials showing preliminary activity.
C. Labile materials.
D. Materials which have passed sequential testing in some other test system.
E. New materials.

LIST OF COMPOUNDS

8-Azaguanine: NSC-749; CAS reg. No. 529-99-7; 7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one, 5-amino-1,6-dihydro-

Azaserine: NSC-742; CAS reg. No. 115-02-6; serine, diazoacetate (ester), L-

BCNU: NSC-409962; CAS reg. 154-93-8; urea, 1,3-bis-(2-chloroethyl)-1-nitroso-

BIC: NSC-82196; CAS reg. No. 5034-77-5; imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-

Cortisone: NSC-9703; CAS reg. No. 53-06-5

Cycloleucine: NSC-1026: CAS reg. No. 52-52-8; cyclopentanecarboxylic acid, 1-amino-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino] tetrahydro-, 2-oxide, monohydrate; Cytoxan

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C

DIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

Dichloromethotrexate: NSC-29630; glutamic acid, *N*-[3,5-dichloro-4-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

5-Fluorouridine: NSC-27640; uridine, 2′-deoxy-5-fluoro-

Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate; 6-MP

Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-GAG: NSC-32946; CAS reg. No. 24968-67-0; guanidine, 1,1′-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate; methylglyoxalbisguanyl hydrazone

Mitomycin C: NSC-26980; CAS reg. No. 50-07-7

NSC-3138: acetamide, *N*,*N*-dimethyl-

NSC-29189: purine-6,8-dithiol, 2-amino-

NSC-38280: CAS reg. No. 18920-92-4; terephthalanilide, 2-chloro-4′,4″-di-2-imidazolin-2-yl-, dihydrochloride

NSC-51845: CAS reg. No. 879-61-8; cyclohexylamine, *N*,*N*-bis(2-chloroethyl)-, hydrochloride

NSC-57155: CAS reg. No. 2053-23-8; terephthalamidine, N′,N‴-bis[*p*-(methylamidino)phenyl]-, tetrahydrochloride

NSC-60339: CAS reg. No. 70-09-7; terephthalanilide, 2-chloro-4′,4″-di-2-imidazolin-2-yl-

6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-

Thioguanine riboside: NSC-29422; CAS reg. No. 85-31-4; 9*H*-purine-6-thiol, 2-amino-9-β-D-ribofuranosyl-

Triethylenemelamine: NSC-9706; CAS reg. No. 51-18-3; 2-triazine, 2,4,6-tris(1-aziridinyl)-

Urethan: NSC-746; ethyl carbamate

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; leurocristine, sulfate (1:1)

GLOSSARY

C	Control group
CCNSC	Cancer Chemotherapy National Service Center
CMC	Carboxymethylcellulose
D	Dilution
DBP	Dibenzpyrene
DCT	Division of Cancer Treatment
DDB	Drug Development Branch
DEB	Drug Evaluation Branch
DMBA	Dimethylbenz(*a*)anthracene
DN	Decision Network
DR&D	Drug Research and Development
ED50	Dose level which inhibits growth to 50% of the control growth
g	Gram
ILAR	Institute of Laboratory Animal Resources
ic	intracranial
im	Intramuscular
ip	Intraperitoneal
iv	Intravenous
kg	Kilogram
LD50	Dose level that is lethal to 50% of the animals treated
LL	Lewis lung carcinoma
MCA	Methylcholanthrene
mg	Milligram
MG&APS	Mammalian Genetics and Animal Production Section
PRA	Program Analysis Branch
sc	Subcutaneous
SD	Standard deviation
ST	Survival time
T	Test group
T/C	Mean or median survival time, diameter, or tumor weight of test group divided by that of the control group
W	Weight
W256	Walker carcinosarcoma 256

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APPENDIX I

Interpretation Chart for Coding Used in DR&D Screening Data Summaries

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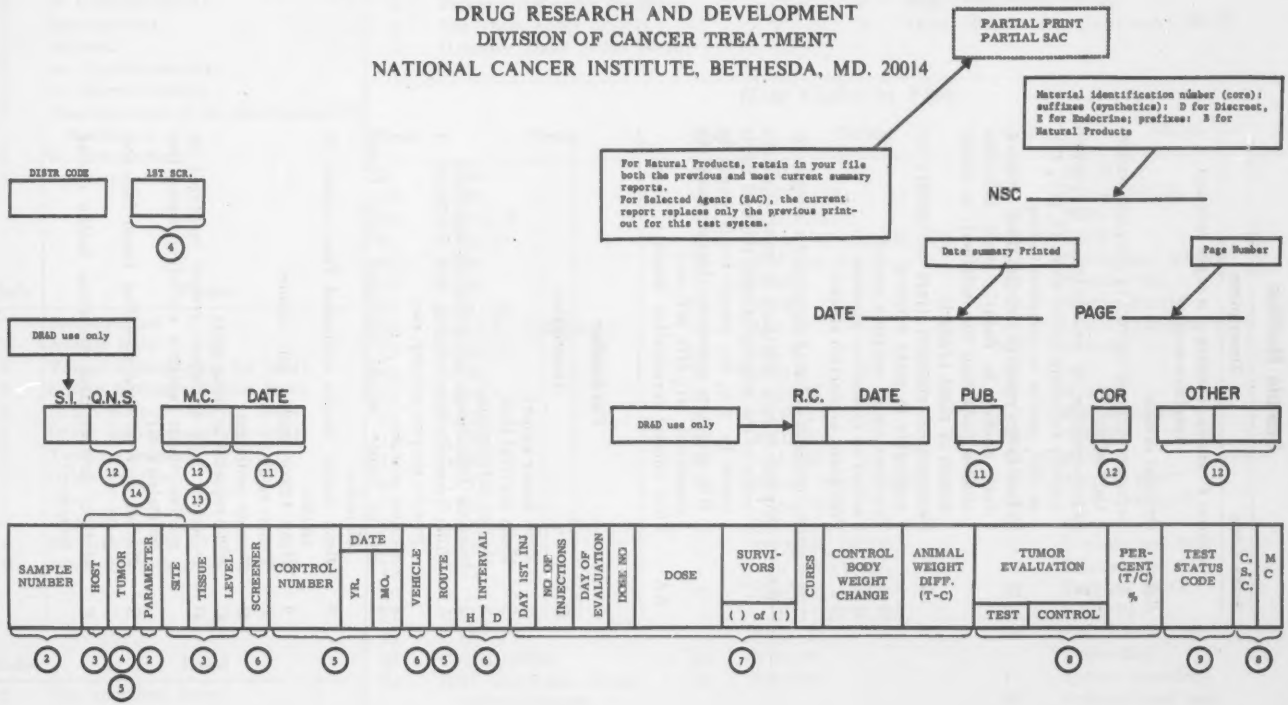
SCREENING DATA SUMMARY INTERPRETATION

DRUG RESEARCH AND DEVELOPMENT
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE, BETHESDA, MD. 20014



NOTE: Numbers in circles (2) refer to page numbers on which interpretations appear.

Sample Number

Suffix Code	Description
	Natural Products: consists of a suffix code and specimen number
None	Original sample
A	Refermentation or extract to complete sequential testing
B	Refermentation or re-extraction to confirm activity following status codes 15, 26P, 27P, or 28P, and/or to complete testing
D	Fermentation research (media, time, temperature studies in flasks), plant collection studies (collection for isolation), or re-collection of plant products
E	Fermentation research (jars or tanks); not applicable in plant extracts
F	Fractionation or isolation studies
K	Purified or crystalline products
J	Pilot plant production studies
	Synthetics: identifies different samples of a compound
MA	Original sample (if there is more than one bottle of the original sample, the first bottle would be MA, the second bottle would be MB, and any subsequent bottles would be numbered: MC, MD, ME, etc)
RA	First clinical formulation tested in vivo

Parameter

Code	Description
1	Mean tumor weight
2	Mean survival time
3	Median survival time
4	Survival time (mean or median not specified)
5	ED50 (concentration causing 50% inhibition of growth, enzyme activity, etc)
6	Alkaloid content
7	Median tumor weight estimated from tumor diameter
8	Mean tumor weight estimated from tumor diameter
9	Mean tumor packed cell volume
A	Tumor volume
B	Percent inhibition
C	ED50 reported in molar units
D	Delay in days of tumor growth measured by median tumor weights excluding tumor-free animals (T–C)
E	Median survival time excluding tumor-free animals
F	Median survival time excluding deaths during treatment

Inoculum

Code	Site
*	Not specified
1	ip (intraperitoneal)
2	sc (subcutaneous)
3	Spontaneous
4	Induced
6	im (intramuscular)
7	ic (intracerebral)
8	Vascular area of the chorioallantoic membrane
9	Iv (intravenous)
A	Not applicable

Code	Tissue
*	Not specified
1	Ascitic fluid
2	Tumor homogenate (or brei)
3	Spleen homogenate (or brei)
4	Brain homogenate (or brei)
5	Other homogenate (or brei)
6	Tumor fragment
7	Other fragment
8	Blood
9	Thymus
A	Not applicable
B	Normal tissue, not specified

Code	Level
*	Not specified, other
1–9	Log of cells, eg, 5 = 10^5 cells
A	Not applicable
B	Dilution 1-2
C	Dilution 1-3
D	Dilution 1-4
E	Dilution 1-5
F	Dilution 1-6
G	Dilution 1-10

Host

Host Group Codes

Code	Group	Code	Group
3	Mouse (host codes 01–49)	8	Egg (host codes 80–85)
5	Rat (host codes 50–69)	9	Other than in vivo (host codes 90–99)
7	Hamster (host codes 70–75)		

Host Codes in Vivo

Code	Strain	Code	Strain	Code	Strain
01	Swiss	25	A/He	46	AL/N
02	BDF_1	26	C57BL/6An	47	BALB/cfC3H
03	C57BL/6	27	A/J (A/Cl)	48	DBA/8
04	DBA/2	28	BALB/cJ (BALB/cSc)	49	$CD8F_1$
05	BCF_1	29	BALB/c An	50	Non-inbred albino
06	CDF_1	30	NZB	51	Fischer 344
07	C3H/He	31	NZW	52	Wistar-Furth
08	$C3AKF_1$ ($CHKRF_1$)	32	SJL/J	53	Lewis (same as 57)
09	BALB/c	33	SM/J	54	Buffalo
10	DBF_1	34	CBA/J	55	ACI
11	CBF_1	35	CAF_1/N	56	Wistar
12	BAF_1	36	CAF_1/J	57	Wistar/Lewis
13	ABF_1	37	$AKD2F_1$	58	OM/N
14	DCF_1	38	$D2AKF_1$	59	M520
15	LAF_1	39	Mouse species not specified	60	August 28807
16	ALF_1			61	ACP (Piebald)
17	$KRCHF_1$ ($AKC3F_1$)	40	$C3D2F_1$	62	Albany
18	AKR/Lw	41	$ZWZBF_1$ (NZW/Bl x NZB/Bl)	63	Copenhagen 2331
19	C57L			64	Zimmerman 61
20	A/L	42	$ZBZWF_1$ (NZB/Bl x NZW/Bl)	65	Yoshida 38366
21	C3Hf			66	NBR/Pl
22	CAF_1	43	CFW/Pl	69	Rat, species not specified
23	C57BL/10SC	44	NIH/Pl	70	Syrian hamster
24	NBL (mutation from C57BL/10H2d)	45	PRI/Pl	80	Embryonated egg

Host Codes Other Than In Vivo

90	Cell culture tube assay	95	Cell-free
91	Chemical analysis	98	Biochemical assay
93	Cell culture chromatography	99	Microbial
94	Bioautography		

Tumor

Code	System	Code	System
AA	Toxicity test (nontumored animals)	HU	L1210/hydroxyurea (NSC-32065)
AC	Carcinoma, adrenal cortex (No. 2)	HX	Hep 2/methotrexate (NSC-740)
AD	ADJ-PC-22 plasma cell	H1	HS1 human sarcoma (rat, egg)
AG	L1210/8-azaguanine (NSC-749)	H2	Hep 2 human epidermoid carcinoma
AK	AKR leukemia (lymphoma)	H3	Hep 3 human epidermoid carcinoma
AL	Alkaloid (chemical analysis)	IC*	L1210 ic inoculation (*see* LE)
AM	Amelanotic melanoma (No. 4)	IC*	Dunning leukemia ic inoculation (*see* DL)
AW	P388/NSC-57155 (a terephthalanilide)	KB	Human epidermoid carcinoma, mouth (cell culture)
A2	ADJ-PC-20 plasma cell	K4	AK4 lymphoid leukemia
A3	Lieberman plasma cell No. 1 (LPC-1)	L[illegible]	L1210/BIC (NSC-82196)
A5	ADJ-PC-5 plasma cell	LC	L1210/cytosine arabinoside (NSC-63878)
BC	L1210/BCNU (NSC-409962)	LD	L1210/DIC (NSC-45388)
B1	B16 melanocarcinoma	LE	L1210 lymphoid leukemia
CA	Adenocarcinoma 755	LF	L1210/methotrexate (NSC-740) + dichloromethotrexate (NSC-29630) [FR-8]
CD	CD8F$_1$ mammary tumor	LL	Lewis lung carcinoma
CH	Chang liver (cell culture)	LM	L1210/dichloromethotrexate (NSC-29630) [M66-3R]
CM	Dunning leukemia/mitomycin C (NSC-26980) (solid)	LP	Liposarcoma (No. 1)
CS	Dunning leukemia/cycloleucine (NSC-1026) (solid)	LW	L1210/NSC-38280 (a terephthalanilide)
C3	C3H mammary tumor	LX	L1210/methotrexate (NSC-740)
DA*	Dunning leukemia (ascitic) (*see* DL)	LZ*	L1210, subcutaneous (*see* LE)
DH	Dunning leukemia/hexamethylmelamine (NSC-13875) (solid)	L2	Leiomyosarcoma (No. 2) (hamster)
DL	Dunning leukemia	L2	Lymphoma 2 (mouse)
DM	DMBA-induced mammary adenocarcinoma	L4	Lymphoma 4
DN	Dunning leukemia/NSC-51845 (a nitrogen mustard) (solid)	L8	L5178Y lymphatic leukemia (mouse)
DR	Dunning leukemia/NSC-29189 (a thiopurine) (ascitic)	L8	Lymphoma 8 (rat)
DX	Dunning leukemia/cyclophosphamide (NSC-26271) (ascitic)	MA	Mammary adenocarcinoma 13762
D1	Adenocarcinoma, duodenum (hamster and cell culture)	MC	Adenocarcinoma, breast
EA	Ehrlich ascites	ME	Mecca lymphosarcoma
EM	Ependymoblastoma	ML	L1210/methyl-GAG (NSC-32946)
EN	Adenocarcinoma, endometrium	MM	Melanotic melanoma
FR	P815/5-fluorouridine (NSC-27640) (ascitic)	MP	L1210/6-mercaptopurine (NSC-755)
FS	Fibrosarcoma (No. 2)	MS	Murphy-Sturm lymphosarcoma
FU	P815/5-fluorouracil (NSC-19893) (ascitic)	M2	MPC-2 plasma cell
FV	Friend virus leukemia (solid)	NH	Novikoff hepatoma
GA	Gardner 6C3HED lymphosarcoma	NP	Plasmacytoma No. 1/BCNU (NSC-409962)
G1	Glioma 261	NR	Neurilemmoma No. 1
G2	Glioma 26	OG	Osteogenic sarcoma
HE	Hepatoma 129 (mouse)	OS	Osteogenic sarcoma He 10734
HE	Cystadenocarcinoma, liver (No. 1) (hamster)	PL	P815/vinblastine (NSC-49842)
HE	HeLa human carcinoma (cell culture)	PM	Plasmacytoma No. 1/triethylenemelamine (NSC-9706)
HR	Hep 2/6-mercaptopurine (NSC-755)	PN	Adenocarcinoma, pancreas (No. 1)
		PR	Adenocarcinoma, prostate
		PS	P388 lymphocytic leukemia
		PT	Carcinoma, pituitary

*Discontinued tumor code.

Systems followed by a slash (/) are resistant to the compound named beyond the slash.

Tumor—Continued

Code	System	Code	System
PV	P388/vincristine (NSC-67574)	XE	[Ehrlich ascites tumor enzymes] (biochemical assay)
PW	P388/NSC-38280 (a terephthalanilide)	XL	[Mouse L1210] (biochemical assay)
PX	Plasmacytoma No. 1/cyclophosphamide (NSC-26271)	XM	[Human leukemia cell enzyme] (biochemical assay)
PY	PY89 sarcoma	XN	[Human erythrocyte enzyme] (biochemical assay)
P1	Plasmacytoma No. 1	XR	[Human RBC (whole)] (biochemical assay)
P4	P1534 leukemia	XS	[Human RBC (unspecified)] (biochemical assay)
P8	P815 mast cell leukemia (ascitic)	XX	[Human RBC (broken)] (biochemical assay)
P9	P329 reticulum cell sarcoma	2X	P288/methotrexate (NSC-740)
RC	Adenocarcinoma, kidney	4A	L4946/azaserine (NSC-742)
RS	Reticulum cell sarcoma (Kelley) (mouse)	5P	P335 leukemia
RS	Reticulum cell lymphosarcoma No. 5 (hamster)	6T	L1210/6-thioguanine (NSC-752)
SA	Sarcoma 180	7P	Ca755/6-mercaptopurine (NSC-755) (solid)
SB	Adenocarcinoma, small bowel	8C	P1798/cortisone (NSC-9703)
TG	Dunning leukemia/thioguanine riboside (NSC-29422)	8P	P1798 lymphosarcoma
WA	Walker carcinosarcoma 256 (subcutaneous)	25	Carcinoma 1025
WC	Walker carcinosarcoma 256/cyclophosphamide (NSC-26271) (subcutaneous)	28	P288 lymphocytic leukemia
WI*	Walker carcinosarcoma 256 (intraperitoneal) (*see* WA)	49	L4946 lymphatic leukemia (solid)
WM*	Walker carcinosarcoma 256 (intramuscular) (*see* WA)	81	P1081 chloroleukemia
WP*	Walker carcinosarcoma 256 (pulmonary) (*see* WA)	91	S91 Cloudman melanoma
		98	C1498 myeloid leukemia

*Discontinued tumor code.

Systems followed by a slash (/) are resistant to the compound named beyond the slash.

Date Experiment Started

Code	Description
Last two digits of calendar year	Year of experiment
Month	
1–9	Jan-Sept
O	Oct
A	Nov
B	Dec

Control Number

Experiment identification number. Numbers are assigned by screening laboratory in numerical order within each test system.

Route of Administration

Code	Description
0	None (controls only)
1	ip (intraperitoneal)
2	sc (subcutaneous)
3	Oral (nonfasting)
4	Other
5	iv (intravenous)
6	im (intramuscular)
7	Oral with prior fasting

Interval (formerly dose regimen)

Time interval between each treatment. If # symbol is used in either Hours or Days columns, the individual codes for hours and days do not apply (*see* Combined Codes below for definition).

Hours		*Days*	
Code	Description	Code	Description
+	Blank	1	Daily (also single)
1–9	Every 1–9 hours	2	Every other day
0	Every 10 hours	3	Every 3rd day
A	Every 11 hours	4	Every 4th day, etc, through—
B	Every 12 hours, etc, through—	9	Every 9th day
M	Every 23 hours	0	Every 10th day
X	Infusion	A	Every 11th day, etc, through—
#	Defined below	E	Every 15th day
		#	Defined below

Combined Codes

Code	Description
#A	Daily, twice a day (hourly interval not specified)
#B	Daily, three times a day (hourly interval not specified)
#C	Ad libitum, water
#D	Ad libitum, diet
##	Other

Screener

Code	Laboratory
01	Microbiological Associates, Inc.
02	Hazleton Laboratories
03	Battelle, Columbus Laboratories
04*	Stanford Research Institute
05	WARF Institute, Inc.
06	A. D. Little, Inc. (in vivo)
07*	Abbott Laboratories
08	Southern Research Institute (in vivo)
09	IIT Research Institute
10*	Charles Pfizer and Co.
11*	Pitman-Moore Co.
12*	Schering Corp.
13*	Wm. S. Merrill Co.
14*	University of Miami (in vivo)
15*	Wyeth Laboratories
16	University of Miami (in vitro)
17	A. D. Little, Inc. (in vitro)
18	Southern Research Institute (in vitro)
19*	Carver Foundation
20*	Sloan-Kettering Institute
21	Cancer Research Institute (Bombay)
22	Central Drug Research Institute (Lucknow)
23	Mason Research Institute
24	Research Triangle Institute
25	The Weizmann Institute of Science (Tel Aviv)
26	The Catholic Medical Center of Brooklyn and Queens, Inc.
27	Institute for Pharmacological Research "Mario Negri" (Milan)
28	Institute Jules Bordet (Brussels)
29	Japanese Foundation for Cancer
30	Bristol Laboratories
31	University of Wisconsin (in vitro)
32	Upjohn Co.
33	University of Alberta (biochemical assay)
34	Yale University (biochemical assay)
35	University of Arizona (in vitro)
40	Screening Section, DEB
41	Associate Chief for Laboratory Research, DR&D
42	Biochemistry Section, DEB
92–99	NCI information only

*Discontinued screener.

Vehicle

Code	Description
0	None
1	Methylcellulose (MC)
2	Saline
3	Acid diluted with saline
4	Steroid suspending solution
5	Alkali diluted with saline
6	Olive oil, sesame oil, peanut oil
7	Other
8	Carboxymethylcellulose (CMC)
9	Water
A	Normal media
B	Propylene glycol
C	Acetone
D	Alcohol
E	Dimethyl formamide
F	Dioxane
G	Dextrose
H	Acid diluted with CMC
K	Lactate
L	Clinical formulation
M	Klucel (hydroxypropylcellulose) (HPC)
P	PVP
Q	Citric acid
R	Lactic acid
S	Saline sonified
T	Saline with Tween-80
U	Gum acacia
V	Sodium bicarbonate
W	Saline + Tween-80 + alkali
X	DMSO
Y	Alkali diluted with CMC
Z	Saline with alcohol

*Discontinued screener.

No. of Injections

Code	Description
01–99	Total No. of injections
A	Ad libitum
Z	Until death

Day of Evaluation

Code (Day)	Description
01–98	Day experiment is ended and survivors are killed
99	Experiment ended on or after Day 99
Z	Discontinued code. Evaluation on day of last death. Day of sacrifice not specified.

Dose No.

Code	Description
0	Single level
1–9	Multi-dose assay
+	10
A	11
B	12
etc	etc

Note: The concentration of the dose decreases as the dose number increases (eg, dose Nos. 1, 2, and 3 = dose concentrations 400, 200, and 100 respectively)

Dose

Doses are reported in mg/kg/injection or dilution per injection, unless otherwise noted. Dilution expressed as a/bcde where a = volume of original material, bcde = final volume.

Code	Description
M	μg/kg
G	g/kg
C	Dilution factor 10^2
K	Dilution factor 10^3
N	1/1000 μg/kg
%	Percent of drug inhaled or (by weight) in feed
U	IU/animal
	Cell culture
W	μg/ml
D	Dilution

Survivors

Number of survivors of total number of animals started on test. (Recorded on Day 5 in survival systems as a measure of drug toxicity.)

Cures

Number of animals with complete inhibition of tumor, or survivors on day of evaluation in survival systems.

Code	No. of cures
0–9	0–9
A	10
B	11
etc	etc

Control Body Weight Change

Average weight change of control animals in grams (weight on toxicity evaluation day minus weight on initial day of treatment)

Animal Weight Difference (T–C)

For survival test systems and Ehrlich ascites: average weight change (weight on toxicity evaluation day minus weight on initial day of treatment) of test group minus average weight change of control group, in grams.

For tumor inhibition systems except Ehrlich ascites: average net weight change (net weight change = gross weight change minus tumor weight) of test group minus average net weight change of control group, in grams.

For egg host system: average weight change of test embryos minus that of control embryos, in grams.

Day of First Injection*

Code	Day
0–9	0–9
A	10
B	11
C	12
etc	etc

*Tumor implanted Day 0.

Control Status Code (CSC)

Code	Description
1	Satisfactory control
2	Excessive deaths
3	Excessive "no-takes"
4	Mean tumor weight or survival time is outside limits
5	Other reasons (contamination, etc)
6	Excessive deaths and excessive "no-takes"
7	Excessive deaths and mean or median survival time or mean tumor weight are outside limits
8	Excessive "no-takes" and mean or median survival time or mean tumor weight are outside limits
9	T/C of positive control is outside limits at standard dose
A	Test of positive control compound at standard dose is toxic in otherwise satisfactory control

Marginal Codes

Code	Data type
	Special Study Testing
A	Comparison study
B	Schedule dependency
C	Combination chemotherapy
J	Comparison-schedule dependency
K	Spontaneous AKR testing
R	Sensitive matching control for resistant tumor experiment
	Other Types
T	Transfer data (a natural product fraction assigned a synthetic NSC number)
X	Corrections to data previously processed (formerly coded "C") (code appears only during week correction is made)
*	New data processed in this week (appears only if no special study code is used)

Evaluation

Test

Mean tumor weight of test animals (T); mean or median survival time of test animals (T).

Control

Mean tumor weight of control animals (C); mean or median survival time of control animals (C).

The units are specified in the individual protocols.

Percent (T/C)

Ratio of tumor weight or survival time of treated animals to control animals, expressed as percent.

Test Status Code

Code	Description
1,3,5,7	Toxic test
2,4,6,8	Nontoxic inactive
11,13	Passed Stage 1 of sequential screen
15	Passed Stage 2 of sequential screen
17	Passed Stage 3 of sequential screen
20	Confirmation testing
21	Single test assay (natural products)
22,24,25	Multi-dose assay (synthetics only)
23	Single test assay (synthetics)
26,27,28	Multi-dose assay (natural products only)
29	Positive control
33	Test to be repeated
34	Test to be repeated, absence of ascitic fluid
35	Published negative data
#	Natural products: test data is on the consolidated file
#*	Natural products: test data is on both the consolidated file and the weekly processing file

Suffix	Description
A	Preliminary confirmation
C	Activity confirmed
E	Exception to routine testing procedure
F	Activity failed criteria
N	Activity not confirmed
P	Activity passed criteria
*Q	Machine-assigned code 20
X	Published negative data (partial data)

*Discontinued.

Cell Culture

ED50: The dose that inhibits growth to 50% of control growth. For materials tested by weight (W in dose column), ED50 is expressed in micrograms per ml. For materials tested by dilution (D in dose column), ED50 is expressed as a dilution, eg, $2.2 \times 10(3)$ equals a dilution of 1:2200. L = less than; M = more than. The number in parentheses is the power of 10.

Slope: Change of response for each 1-log change of dose.

Biochemical Testing

ED50: Reported in molar concentrations. L = less than; M = more than. The number in parentheses is the power of 10. S = strong (10^{-4} M or less); M = moderate (10^{-4} – 10^{-5} M); W = weak (10^{-5} – 10^{-4} M).

Percent inhibition: S = strong* (76%–100%); M = moderate* (51%–75%); W = weak* (26%–50%); I = inactive* (0–25%); E = enhancement* (negative percent); A = followup testing.

Metabolic pathway or enzyme:

00 = Purine synthesis de novo (Purine)
10 = Adenine phosphoribosyltransferase (APT)
20 = Adenosine kinase (AK)
30 = Inosine synthesis (INOS)
50 = Hypoxanthine, guanine phosphoribosyltransferase (HGPT)
60 = Dihydrofolic reductase (DHFR)

*Rate at 1 millimol.

Evaluation

I. Survival Tumor Systems

Evaluation on the basis of survival (parameters 2, 3, 4).

In general, a minimal increase in survival time of treated animals over control animals resulting in a T/C $\geq$ 125% is necessary for further experimental work.

II. Tumor Inhibition Systems

Evaluation on the basis of tumor inhibition (parameters 1, 8, 9, A).

In general, a minimal reproducible tumor inhibition of treated animals over control animals resulting in a T/C $\leq$ 42% is necessary for further experimental testing.

III. In vitro activity indicative of a significant biological effect is used as a basis for requesting the larger amounts needed for further testing to determine selective toxicity against tumors in vivo. For crude natural products with demonstrated in vivo activity, in vitro testing is used, when possible, for biological assay during fractionation leading to isolation of the antitumor material.

IV. Criteria for Decision Network Evaluation (*see* pages 12 and 13 of Appendix I for MC codes)

In subsequent experimental testing, activities substantially greater than the minimal end points described above are generally required. Criteria for acceptance as a candidate for development toward clinical trial are dependent on a number of factors, including the predictive value and overall sensitivity of the test system as well as on the nature and physical-chemical properties of the test material. With the further accumulation of both laboratory and clinical results, these criteria may be reviewed periodically by the Division of Cancer Treatment staff, NCI and are flexible. Suppliers are notified by letter of actions taken which are reflected by MC codes 1 to 4.

Alkaloid System

Approximate weight of dried plant in kilograms to yield 1 gram of alkaloid.

Code	Description
0	Negative or trace amount of alkaloid
1	10 kg
2	3 kg
3	1 kg
4	< 1 kg

Evaluation Code—Natural Products

Code	Description
Blank	has not been evaluated
C	A material which has passed DR&D confirmation protocols in the test system designated
D	A former "C" which is no longer of interest and has been dropped from further testing in the confirmed system (WS 23 received)
F	A material which has failed confirmation testing in the test system designated
S	To be isolated in system listed although previously confirmed in another system
U	A material in fractionation testing even though it has not passed confirmation in test system designated
X	Instruction to discontinue testing was received before routine testing was completed

Publication Code (PUB)

Code	Description
0 or Blank	Not published
1	Published in *Cancer Research Supplement* (*CRS*)
*2	Published in *CRS* with source
*3	Published in *CRS* without source
*4	Published in *Cancer Chemotherapy Reports* (*CCR*) with source
*5	Published in *CCR* without source
*6	Publishable with source
*7	Publishable without source

*Not yet implemented.

Date

Code	Description
MC	Date Material Classification Code assigned: year, month
EVAL	Date Evaluation Code assigned: year, month
SCHED	Date Material Shipping List processed: year, month
YR	Last two digits of calendar year
MO	
1–9	Jan–Sept
0	Oct
A	Nov
B	Dec

Evaluation Code—Synthetics

Code	Description
Blank	Has not been evaluated
A	Basis for assignment to 2A
B	Basis for assignment to 2B
N	Failed (MC code 1) criteria
E	Results equivocal or testing inadequate
1	Meets (MC code 1) criteria
2	Meets DN–2 criteria

Quantity Not Sufficient (QNS)

Code	Description
J	No more compound available from original supplier
K	Refill requested
L	On list for preparation laboratory
Q	Compound no longer available from original supplier; activity does not warrant procuring an additional supply
S	Quantity sufficient for cell culture testing only
T	Quantity sufficient for one schedule only
Y	Refill of duplicate (may or may not be a former QNS)
Z	Refill available and cleared but not yet shipped

Correction (COR)

A "C" will appear in this block when corrections are made to data previously processed. An "X" appears to the right of the status code indicating those data lines which were corrected. (Code appears only during week correction is made.)

Other Codes

Code	Description
AT	Plant and animal materials formerly assigned synthetic numbers (test data moved to natural products file)
ET	Endocrine testing
NT	No testing processed
ST	Special testing

Material Classification (MC)

Natural Products

Code	Description
C	A material which has passed DR&D confirmation protocols in one or more tumor systems
CC	A "C" from which a purified active material has been isolated
D	A former "C" which is no longer of interest and has been dropped from further testing in the confirmed system (WS 23 received)
DA	Deferred due to inability to recover active principle
DD	Work on all collections of this genus and species considered complete
DK	Deferred for presence of known compound
DL	Deferred because culture of fermentation product was lost or re-collection of plant or animal not available
DM	Deferred due to absence of activity in L1210, P388, or W256
DN	Deferred for insufficient activity
DR	Deferred for failure to reconfirm
DS	Deferred due to instability of active principle
DT	Deferred due to excessive toxicity
D1	First re-collection inactive
D2	Second re-collection inactive
F	A material which has failed confirmation testing in one or more test systems
N	A material which has failed confirmation with testing completed in all scheduled test systems
S	To be isolated in system listed although previously confirmed in another system
U	A material in fractionation testing even though it has not passed confirmation
UC	A "U" from which a purified active material has been isolated
V	A dropped "U"
VK	A "V" from which a known purified active material has been isolated
X	Worksheet 23 to discontinue testing has been received prior to completion of routine testing

Material Classification (MC)—Continued

Synthetics (see page 15)

Code	Description
*	Formerly listed as Selected Agent
X	Testing at time of review considered incomplete. No folder available.
0	Special interest or folder available.
1	Reproducible minimal activity: T/C $\geq$ 125%/$\leq$ 42% (2 controls, same lab or 2 labs)
D	Deferred: does not meet DN-2 activity criteria
2	Decision Network (DN)-2A
A	Passed: DR&D test system
B	Dropped: not superior to parent compound or DEC decision point
C	Passed: "other" systems
D	Dropped: failed activity criteria
E	Passed: endocrine compound
R	Recycle
	DN-2B
F	Dropped: production or usable formulation not feasible
P	Passed: go to 3
R	Recycle
S	Dropped: insufficient activity in route or schedule dependency study
3	DN-3: toxicology
A	Passed 3: file IND, go to DN-4
F	Dropped: formulation not feasible
R	Recycle
T	Dropped: toxicology
4	DN-4: IND filed
A	Passed: go to DN-5
B	Passed 4: bypass DN-5, go to DN-6
D	Dropped: irreversible toxicity in man
R	Recycle
5	DN-5
A	Passed: effective in man, go to DN-6
D	Dropped: IND withdrawn
T	Dropped: IND withdrawn due to toxicity in man
6	DN-6
A	Passed: effective in man, go to DN-7
D	Dropped: not effective in man (IND withdrawn [negative] after clinical trial)
R	Recycle
7	DN-7 NDA
A	NCI development
B	Not NCI development
E	Endocrine compound

Test Systems
(examples of host, tumor, parameter, site)*

3AA	3B139	3FR41	3LE31	3L431	3SAD2
3AA2	3B172	3FU21	3LE32	3L831	3SAE2
3AA3	3CAD2	3FV12	3LE39	3L841	3SA12
3AA4	3CAE2	3GA31	3LF32	3ME41	3SA32
3AD12	3CA12	3GA41	3LLD2	3ML21	3SA72
3AG21	3CA32	3G112	3LLE2	3MP21	3SA82
3AK31	3CA72	3G137	3LL12	3M212	32X31
3AK33	3CA82	3G212	3LL16	3OS12	32512
3AK39	3CD12	3G237	3LL31	3PL31	32841
3AKF3	3CD13	3HE12	3LL32	3PS21	34A22
3A212	3CD32	3HU21	3LL33	3PS31	34922
3A331	3CD33	3HU31	3LL36	3PS37	35P21
3A512	3C3D2	3K431	3LL72	3PV31	36T21
3BC21	3C3E2	3LB31	3LL76	3PW31	37P12
3B1D2	3C332	3LC21	3LL86	3PY12	38C12
3B1E2	3C372	3LD31	3LM32	3P421	38P12
3B112	3EA11	3LE12	3LW21	3P831	38P22
3B131	3EM12	3LE21	3LX21	3P841	38121
3B132	3EM32	3LE27	3LX32	3P931	39112
3B136	3EM37	3LE29	3L221	3RS31	39831
3B137					
5AA	5DL31	5DR31	5MA32	5WA12	5WA31
5AA4	5DL32	5DX31	5MS16	5WA16	5WA46
5CM42	5DL37	5H112	5NH12	5WA21	5WA47
5CS42	5DM1	5H312	5TG42	5WA27	5WC12
5DH42	5DN42	5L822			
7AA	7EN12	7MC12	7OGD2	7PM12	7P112
7AA4	7FS12	7MM12	7OGE2	7PN12	7RC12
7AC12	7HE12	7NP12	7OG12	7PR12	7RS12
7AM12	7LP12	7NR12	7OG32	7PT12	7SB12
7D112	7L212	7OGD2	7OG72	7PX12	
8H118					
9AL6	9D15	9HR5	9HX5	9H25	9KB5
9CH5	9HE5				

Biochemical Systems

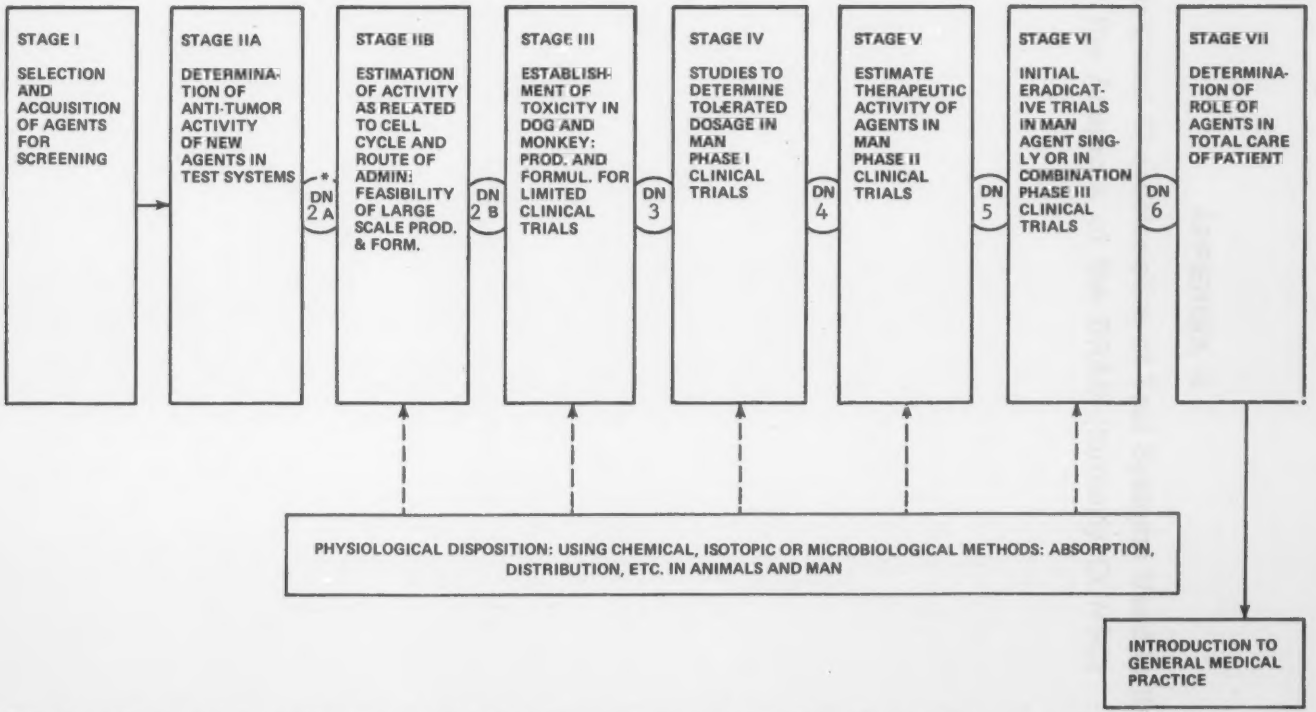
9XEB	9XMC	9XNC	9XRB	9XSB	9XXB
9XLC					

*These combinations of codes are those currently in use (1972).

DIVISION OF CANCER TREATMENT LINEAR ARRAY

(Summary)

NEW DRUG DEVELOPMENT FLOW


STAGE I
SELECTION AND ACQUISITION OF AGENTS FOR SCREENING
STAGE IIA
DETERMINATION OF ANTI-TUMOR ACTIVITY OF NEW AGENTS IN TEST SYSTEMS
*
DN 2 A
STAGE IIB
ESTIMATION OF ACTIVITY AS RELATED TO CELL CYCLE AND ROUTE OF ADMIN: FEASIBILITY OF LARGE SCALE PROD. & FORM.
DN 2 B
STAGE III
ESTABLISHMENT OF TOXICITY IN DOG AND MONKEY: PROD. AND FORMUL. FOR LIMITED CLINICAL TRIALS
DN 3
STAGE IV
STUDIES TO DETERMINE TOLERATED DOSAGE IN MAN PHASE I CLINICAL TRIALS
DN 4
STAGE V
ESTIMATE THERAPEUTIC ACTIVITY OF AGENTS IN MAN PHASE II CLINICAL TRIALS
DN 5
STAGE VI
INITIAL ERADICATIVE TRIALS IN MAN AGENT SINGLY OR IN COMBINATION PHASE III CLINICAL TRIALS
DN 6
STAGE VII
DETERMINATION OF ROLE OF AGENTS IN TOTAL CARE OF PATIENT
PHYSIOLOGICAL DISPOSITION: USING CHEMICAL, ISOTOPIC OR MICROBIOLOGICAL METHODS: ABSORPTION, DISTRIBUTION, ETC. IN ANIMALS AND MAN
INTRODUCTION TO GENERAL MEDICAL PRACTICE

* DN, Decision Network-Full Linear Array in Summary Report of the Chemotherapy Program, NCI, vol 2, March 1972.

[From Summary Report of the Chemotherapy Program, NCI, 1972. HEW, PHS, NIH, NCI, March 1972.]

APPENDIX II

Summary of the Usual Characteristics of Test Systems Used in the Past Under the Auspices of the DR&D (formerly CCNSC)

APPENDIX II.—Summary of the Usual Characteristics of Test Systems Used in the Past

Test system code*	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
3AA4	Nontumored mice (toxicity test)	—	—	—
5AA4	Nontumored rats (toxicity test)	—	—	—
7AC12	Carcinoma, adrenal cortex (No. 2)	Syrian hamsters	10–12	10–12
3AD12	ADJ-PC-22 plasma cell	BALB/c mice	27	27
3AG21	L1210/8-azaguanine (NSC-749)	DBA/2 mice	6–7	6–7
3AK3	Spontaneous AKR lymphoma	AKR mice	—	—
3AK31	AKR lymphoma (transplanted)	AKR mice	When spleen weighs ≥ 600 mg	When spleen weighs ≥ 600 mg
7AM12	Amelanotic melanoma (No. 4)	Syrian hamsters	7	7
3AW31	P388/NSC-57155 (a terephthalanilide)	DBA mice	6–7	7
3A212	ADJ-PC-20 plasma cell	BALB/c mice	21	21
3A331	Lieberman plasma cell No. 1 (LPC-1)	BALB/c or CAF_1 mice	14–15	14–15
3A512	ADJ-PC-5 plasma cell	BALB/c mice	21	21
3B131	B16 melanocarcinoma (*see* Protocol 1)	C57BL/6 mice	10–12	10–12
3B132	B16 melanocarcinoma (*see* Protocol 1)	C57BL/6 mice	10–12	10–12
3CA12	Adenocarcinoma 755	C57BL/6 mice	14	10–14
9CH5	Chang liver (cell culture)	—	—	—
5CM42	Dunning leukemia/mitomycin C (NSC-26980) (solid)	Fischer rats	NA	NA

NOTE: — = Not applicable. NS = not specified. NA = detailed information not available at DR&D.

*Identification code: first symbol (digit) = host group; second and third symbols (letters or letter and digit) = tumor; fourth symbol (digit) = parameter; fifth symbol (digit or letter) = inoculum site. *See* Appendix I (pp 69–85) for definitions of all codes. Systems are listed in alphabetical order by tumor code.

†*See* Appendix I (p 76) for full names of laboratories.

‡ST = survival time.

Testing strain	Testing (tumor) inoculum: Site of implantation	Tissue	Level	Duration of drug administration (non-endocrines were all given ip qd except as noted)
—	—	—	—	Same as parallel test animals with tumor
—	—	—	—	Same as parallel test animals with tumor
Syrian hamsters	Sc	Fragment	—	Days 1–7
BALB/c mice	Sc	Fragment	—	Days 1–10
BDF_1 mice	Ip	Ascitic fluid	NS	Days 1–9
AKR mice	—	—	—	Optimal schedule of age
AKR mice	Ip	Spleen brei	1:10	Days 1–9
Syrian hamsters	Sc	Fragment	—	Days 1–7
BDF_1 or CDBA mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
BALB/c mice	Sc	Fragment	—	Days 1–10
CAF_1 or BALB/c mice	Ip	Ascitic fluid	10^5 cells	Start between Days 14 a 19 and continue for days
BALB/c mice	Sc	Fragment	—	Days 1–10
BDF_1 mice	Ip	Tumor brei	1:10	Days 1–9
BDF_1 mice	Sc	Tumor brei or fragment	1:10	Days 1–9
BDF_1 mice	Sc	Fragment	—	Days 1–11
—	—	—	—	Days 1–3 or 1–4
Fischer rats	Sc	Fragment	—	Day 7 only (sc)

Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
ne as parallel test in nimals with tumor	Same as parallel test in animals with tumor or Day 5	Same as parallel test in animals with tumor	ST ‡	—
ne as parallel test in nimals with tumor	Same as parallel test in animals with tumor or Day 10	Same as parallel test in animals with tumor	ST	—
s 1–7	12	12	Weight	NA
s 1–10	5	27	Weight	1.5–3.0 g
s 1–9	5	30	Mean ST	12–18 days
imal schedule of agent	5	60	Median ST	12–20 days
s 1–9	5	60	Median ST	17–21 days
s 1–7	8	8	Weight	7–10 g
s 1–10	5	30	Median ST	9–14 days
s 1–10	5	21	Weight	1–2 g
rt between Days 14 and 9 and continue for 28 ays	17	45	Median ST	20–28 days
s 1–10	5	21	Weight	1.9–3.5 g
s 1–9	5	60	Median ST	14–22 days
s 1–9	5	60	Median ST	NA
s 1–11	12	12	Weight	0.5–2.0 g
s 1–3 or 1–4	—	3–4	ED50	—
7 only (sc)	NA	Death	ST	NA

table n or control ult	Positive control compound			One of the screening laboratories which utilized this system † (code)
	NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
—	—	—	—	
—	—	—	—	
	742	10	≤ 42	Southern (08)
g	755	12.5	≤ 42	MAI (01)
ays	740	0.75	≥ 135	Southern (08)
ays	26271	150, Day 1 only	≥ 135	Southern (08)
ays	—	—	≥ 135	IITRI (09)
	740	8	≤ 42	Southern (08)
ys	NS	NS	≥ 135	ADL (06)
	755	25	≤ 42	MAI (01)
ays	26271	5	≥ 135	MAI (01)
g	NA	NA	≤ 42	MAI (01)
ays	19893 26271	20 50	≥ 135	
	26271	50	≥ 135	Southern (08)
g	755 749	4 50	≤ 42	
—	NS	—	—	ADL (06)
	NA	NA	≥ 135	Miami U (14)

Test system code[a]	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
5CS42	Dunning leukemia/cycloleucine (NSC-1026) (solid)	Fischer rats	NA	NA
	Dibenzpyrene-induced fibrosarcoma (mouse)	—	—	—
5DH42	Dunning leukemia/hexamethylmelamine (NSC-13875) (solid)	Fischer rats	NA	NA
5DL31	Dunning leukemia (ascitic)	Fischer rats	7	7
5DL32	Dunning leukemia (solid)	Fischer rats	10	10
5DL37	Dunning leukemia (ic)	Fischer rats	7	7
5DM1A	DMBA-induced mammary adenocarcinoma	NA	—	—
5DN32	Dunning leukemia/NSC-51845 (a nitrogen mustard) (solid)	Fischer rats	NA	NA
5DR31	Dunning leukemia/NSC-29189 (a thiopurine) (ascitic)	Fischer rats	NA	NA
5DX31	Dunning leukemia/cyclophosphamide (NSC-26271) (ascitic)	Fischer rats	8–11	10
7D112	Adenocarcinoma, duodenum	Syrian hamsters	7–8	8
9D15	Adenocarcinoma, duodenum	—	—	—
3EA11	Ehrlich ascites	Swiss mice	7	7
3EM37	Ependymoblastoma	Sc in C57BL/6 male mice	10–14	12–14
3EM32	Ependymoblastoma	Sc in C57BL/6 male mice	10–14	12–14
3EM12	Ependymoblastoma	Sc in C57BL/6 male mice	10–14	12–14

Testing strain	Testing (tumor) inoculum: Site of implantation	Tissue	Level	Dur...
Fischer rats	Sc	Fragment	—	Day 7 o...
C57BL/6 or ICR/Ha female mice	Sc	DBP	0.5 mg	Days 1–...
Fischer rats	Sc	Fragment	—	Day 7 o...
Fischer rats	Ip	Ascitic fluid	6	Days 1–5
Fischer rats	Sc	Fragment	—	Days 1–5
Fischer rats	Ic	Ascitic fluid	NS	Days 1–5
NA	—	DMBA	NA	NA
Fischer rats	Sc	Fragment	—	Day 7 on...
Fischer rats	Ip	Ascitic fluid	10^6 cells	Days 1–5
Fischer rats	Ip	Ascitic fluid	10^6 cells	Days 1–5
Syrian hamsters	Sc	Fragment	—	Days 1–5
—	—	—	—	Days 1–3
Swiss mice	Ip	Ascitic fluid	10^6 cells	Days 1–7
C57BL/6 male mice	Ic	Fragment	—	Days 1–5
C57BL/6 male mice	Sc	Fragment	—	Days 1–5
C57BL/6 male mice	Sc	Fragment	—	Days 1–5

Level	Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Accep media mean c resu
—	Day 7 only (sc)	NA	Death	ST	N
0.5 mg	Days 1–10	12	12	*P* value (probability of specificity)	
—	Day 7 only (sc)	NA	Death	ST	N
6	Days 1–5	5	30	Median ST	8–11 da
—	Days 1–5	5	30	Median ST (cures)	12–16 d
NS	Days 1–5	5	30	Median ST	
NA	NA	NA	NA	Weight	N
—	Day 7 only (sc)	NA	Death	Median ST	N
10⁶ cells	Days 1–5	5	Death	Median ST	8–12 da
10⁶ cells	Days 1–5	5	Death	Median ST	N
—	Days 1–5	6	6	Weight	0.4–1.6
—	Days 1–3 or 1–4	—	3–4	ED50	
10⁶ cells	Days 1–7	12	12	Weight of ascitic volume	≥4.5 g
—	Days 1–5	5	5	Median ST	17–21 d
—	Days 1–5	5	5	Median ST	17–21 d
—	Days 1–5	10	10	Weight	0.3–1.0

Acceptable median or mean control result	Positive control compound			One of the screening laboratories which utilized this system † (code)
	NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
NA	NA	NA	≥ 135	Miami U (14)
0	26271	45	$P \geq 10$ for 5 mice	ADL (06)
NA	NA	NA	≥ 135	Miami U (14)
–11 days	26271 755	10 20	≥ 135	ADL (06)
2–16 days	755	20	≥ 135	ADL (06)
NA	755	40	≥ 135	ADL (06)
NA	NA	NA	NA	Southern (08)
NA	NA	NA	NA	Miami U (14)
–12 days	26271	10	≥ 135	ADL (06)
NA	755	20	≥ 135	ADL (06)
.4–1.6 g	26271	50	≤ 42	Southern (08)
—	NS	—	—	Southern (08)
≥4.5 g	7365 3051	0.03 100	≤ 42	Southern (08)
7–21 days	409962	4	≥ 135	
7–21 days	409962	4	≥ 135	Hazleton (02)
.3–1.0 g	409962	4	≤ 42	

Test system code*	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
7EN12	Adenocarcinoma, endometrium	Syrian hamsters	10–12	10–12
3FR41	P815/5-fluorouridine (NSC-27640) (ascitic)	CDBA mice	7	7
7FS12	Fibrosarcoma (No. 2)	Syrian hamsters	NA	NA
3FU31	P815/5-fluorouracil (NSC-19893) (ascitic)	CDBA mice	7	7
3FV12	Friend virus leukemia (solid)	BDF_1, DBA_2, Swiss mice	14	13–14
3GA41	Gardner 6C3HED lymphosarcoma	C3H mice	7	7
3G137	Glioma 261	Sc in C57BL/6 male mice	10–14	12–14
3G112	Glioma 261	Sc in C57BL/6 male mice	10–14	12–14
3G237	Glioma 26	Sc in C57BL/6 male mice	10–14	12–14
3G212	Glioma 26	Sc in C57BL/6 male mice	10–14	12–14
3G282	Glioma 26	NA	NA	NA
3HE12	Hepatoma 129 (mouse)	C3H/He mice	14	14
7HE12	Cystadenocarcinoma, liver (No. 1) (hamster)	Syrian hamsters	NA	NA
9HE5	HeLa human carcinoma (cell culture)	—	—	—
9HR5	Hep 2/6-mercaptopurine (NSC-755)	—	—	—
9HX5	Hep 2/methotrexate (NSC-740)	—	—	—
5H112	HS1 human sarcoma (rat)	Wistar rats	10–11	10–11
8H118	HS1 human sarcoma (egg)	—	—	—

Testing strain	Testing (tumor) inoculum			
	Site of implantation	Tissue	Level	
Syrian hamsters	Sc	Fragment	—	Days 1
BDF_1 or BALB/c mice	Ip	Ascitic fluid	NS	Days 1
Syrian hamsters	Sc	Fragment		Days 1
BALB/c or BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1
BDF_1, DBA_2, Swiss mice	Sc	Fragment	—	Days 1
$CHKRF_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1
C57BL/6 male mice	Ic	Fragment	—	Days 1
C57BL/6 male mice	Sc	Fragment	—	Days 1
C57BL/6 male mice	Ic	Fragment	—	Days 1
C57BL/6 male mice	Sc	Fragment	—	Days 1
NA	Sc	Fragment	—	NA
C3H/He mice	Sc	Fragment	—	Days 5–1
Syrian hamsters	Sc	Fragment	—	Days 1–7
—	—	—	—	Days 1–3
—	—	—	—	Days 1–3
—	—	—	—	Days 1–3
Wistar rats	Sc	Brei	1:2	Days 1–1
—	Chorioallantoic membrane	Fragment	—	Days 1–3

Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
Days 1–11	12	12	Weight	0.8–3.5 g
Days 1–15	5	Death	ST	10–14 days
Days 1–7	12	12	Weight	NA
Days 1–15	5	Death	Mean ST	10–14 days
Days 1–11	12	12	Weight	0.5–2.0 g
Days 1–9	5	30	Median ST	9–13 days
Days 1–5	5	40	Median ST	22–26 days
Days 1–5	10	10	Weight	0.3–1.0 g
Days 1–5	5	40	Median ST	22–26 days
Days 1–5	10	10	Weight	0.3–1.0 g
NA	14	21 days	Tumor diameter	NA
Days 5–14	15	15	Weight	0.5–2.0 g
Days 1–7	8	8	Weight	0.5–2.0 g
Days 1–3 or 1–4	—	3–4	ED50	—
Days 1–3 or 1–4	—	3–4	ED50	—
Days 1–3 or 1–4	—	3–4	ED50	—
Days 1–12	13	13	Weight	10–20 g
Days 1–3 or 1–4	—	9 or 10	Weight	0.5–2.0 g

Positive control compound			One of the screening laboratories which utilized this system † (code)
NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
26271	25	≤ 42	Pfizer (10) Southern (08)
NA	NA	≥ 135	Southern (08)
26271	15	≤ 42	Southern (08)
755	10	≥ 135	Southern (08)
755 26271	20 25	≤ 42	Battelle (03) Southern (08)
740	1	≥ 135	ADL (06)
409962	4	≥ 135	
409962	4	≤ 42	
409962	4	≥ 135	
409962	4	≤ 42	
NA	NA	≤ 75	Sloan-Kettering (20) Southern (08)
3051	200	≤ 42	Hazleton (02) Battelle (03)
740	18	≤ 42	Southern (08)
NS	NS	—	Bristol (30)
NS	NS	—	Southern (08)
NS	NS	—	Southern (08)
45383	25 μg/kg/injection	≤ 42	Sloan-Kettering (20)
26271	500 μg/egg	≤ 42	Merck, Sharp & Dohme Laboratories

Test system code*	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
9H25	Hep 2 human epidermoid carcinoma	—	—	—
5H312	Hep 3 human epidermoid carcinoma	Irradiated rats	10–11	10–11
9KB5	Human epidermoid carcinoma, mouth	—	—	—
3LB31	L1210/NSC-82196 (BIC)	DPB/2 mice	7	7
3LD31	L1210/NSC-45388 (DIC)	DBA/2 mice	7	7
3LE21	L1210 lymphoid leukemia (*see* Protocol 1.100)	DBA/2 mice	7	6–7
3LE31	L1210 lymphoid leukemia	DBA/2 mice	7	6–7
3LE32	L1210 lymphoid leukemia	DBA/2 mice	7	6–7
3LE27	L1210 lymphoid leukemia	DBA/2 mice	7	6–7
3LE29	L1210 lymphoid leukemia	DBA/2 mice	7	6–7
3LL16	Lewis lung carcinoma (*see* Protocol 1.400)	C57BL/6 mice	14	12–14
3LL36	Lewis lung carcinoma	C57BL/6 mice	14	12–14
3LL32	Lewis lung carcinoma	C57BL/6 mice	14	14
3LL12	Lewis lung carcinoma	C57BL/6 mice	14	14
7LP12	Liposarcoma (No. 1)	Syrian hamsters	NA	NA
3LW21	L1210/NSC-38280 (a terephthalanilide)	DBA/2 mice	6–7	6–7
3LX21	L1210/methotrexate (NSC-740)	DBA/2 mice	7	6–7

Testing strain	Testing (tumor) inoculum			Duration of administration (non-endocrine were all given ip qd except as
	Site of implantation	Tissue	Level	
—	—	—	—	Days 1–3 or 1
Irradiated rats	Sc	Brei	1:2	Days 1–8
—	—	—	—	Days 1–8 or 1
BDF_1 mice	Ip	Ascitic fluid	10^5 cells	Optimum sched
BDF_1 mice	Ip	Ascitic fluid	10^4 cells	Optimum sched
BDF_1 mice	Ip	Ascitic fluid	10^5 cells	Day 1 to deat 1–9
BDF_1 mice	Ip	Ascitic fluid	10^5 cells	Days 1–9
BDF_1 or CDF_1 mice	Sc	Spleen brei or Ascitic fluid	10^6 cells	Day 7 or 8 to
BDF_1 or CDF_1 mice	Ic	Ascitic fluid	10^4 cells	Days 1–9
BDF_1 mice	Iv	Ascitic fluid	10^7 cells	Days 1–5
BDF_1 mice	Im	Tumor brei	2×10^6 cells	Days 1–11
BDF_1 mice	Im	Tumor brei	2×10^6 cells	Days 1–11
BDF_1 mice	Sc	Fragment	—	Optimal regime drug for esta mor
BDF_1 mice	Sc	Fragment	—	Days 1–11
Syrian hamsters	Sc	Fragment	—	Days 1–7
BDF_1 mice	Ip	Ascitic fluid	10^5 cells	Days 1–10
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Day 1 to death 1–10

	Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
	Days 1–3 or 1–4	—	3–4	ED50	—
	Days 1–8	Day of evaluation	9–11	Weight	3.6 ± 2.2 g
	Days 1–3 or 1–4	—	3–4	ED50	6-fold growth
s	Optimum schedule	5	30	Median ST	9–11 days
s	Optimum schedule	5	30	Median ST	9–11 days
s	Day 1 to death or Days 1–9	5	30	Mean ST	8–11 days
s	Days 1–9	5	30	Median ST	8–11 days
s	Day 7 or 8 to death	5	30	Median ST	8–11 days
s	Days 1–9	5	30	Mean ST	8–11 days
s	Days 1–5	5	30	Mean ST	8–11 days
s	Days 1–11	12	12	Weight	0.5–2.5 g
s	Days 1–11	5	40	Median ST	18–28 days
	Optimal regimen for each drug for established tumor	NS	NS	Median ST	NS
	Days 1–11	12	12	Weight	0.5–2.0 g
	Days 1–7	8	8	Weight	0.6–2.0 g
s	Days 1–10	5	30	Mean ST	8–11 days
s	Day 1 to death and Days 1–10	5	30	Mean ST	8–11 days

eptable lian or control esult	Positive control compound			One of the screening laboratories which utilized this system † (code)
	NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
—	NS	NS	—	Southern (08)
2.2 g	NS	NS	≤ 42	Sloan-Kettering (20)
growth	755	—	ED50 = 0.01-0.9 μg/ml	
lays	NA	NA	≥ 135	MAI (01)
lays	NA	NA	≥ 135	MAI (01)
lays	19893 749	20 50	≥ 135	
lays	749 740	50 2	≥ 135	
lays	749 740	50 3	≥ 135	
lays	63878 409962		≥ 135	Hazleton (02) and Southern (08)
lays	26271	20	≥ 135	Hazleton (02)
5 g	26271	20	≤ 42	
days	26271	25	≥ 135	
	95441	NS	≥ 135	Southern (08)
g	26271	25	≤ 42	
g	740	15	≤ 42	Southern (08)
lays	740	1.5	≥ 135	ADL (06)
lays	26271	25	≥ 135	Southern (08)

Test system code*	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
7L112	Leiomyosarcoma (No. 1)	Syrian hamsters	NA	NA
3L221	Lymphoma 2 (mouse)	NA	NA	NA
7L212	Leiomyosarcoma (No. 2) (hamster)	Syrian hamsters	NA	NA
3L431	Lymphoma 4	CDBA mice	7	7
3L831	L5178Y lymphatic leukemia (mouse)	BDF_1 or DBA_2 mice	7	7
5L822	Lymphoma 8 (rat)	Lewis rat	11	11
7MC12	Adenocarcinoma, breast	Syrian hamsters	NA	NA
3ME41	Mecca lymphosarcoma	AKR/N mice	7	7
3ML21	L1210/methyl-GAG (NSC-32946)	BDF_1 mice	7	7
7MM12	Melanotic melanoma	Syrian hamsters	14	14
3MP21	L1210/6-mercaptopurine (NSC-755)	DBA/2 mice	NA	NA
5MS16	Murphy-Sturm lymphosarcoma	Non-inbred albino Sprague-Dawley rats	12–14	12–14
3M212	MPC-2 plasma cell	BALB/c mice	14	14
5NH12	Novikoff hepatoma	Non-inbred albino Sprague-Dawley rats	NA	NA
7NP12	Plasmacytoma No. 1/BCNU (NSC-409962)	Syrian hamsters	NA	NA
7NR12	Neurilemmoma No. 1	Syrian hamsters	NA	NA
7OG12	Osteogenic sarcoma	Syrian hamsters	NA	NA
3OS12	Osteogenic sarcoma He 10734	C3H/He mice	20–21	18–20
3PL31	P815/vinblastine (NSC-49842)	CDBA mice	7	7

Testing strain	Testing (tumor) inoculum			Duration of administration (non-endocrine were all ip qd except as
	Site of implantation	Tissue	Level	
NA	Sc	Fragment	—	NA
CAF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
Syrian hamsters	Sc	Fragment	—	Days 1–7
CDBA or CAF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–9
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
Lewis rat	Sc	Wbc	10^6 cells	Days 1–5
Syrian hamsters	Sc	Fragment	—	Days 1–7
$CHKRF_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
BDF_1 mice	Sc	Spleen brei	10^6 cells	Day 1 to death
Syrian hamsters	Sc	Fragment	—	Days 1–7
BDF_1 mice	Ip	Ascitic fluid	NA	Days 1–15
Non-inbred albino Sprague-Dawley rats	Im	Tumor brei	10^6 cells	Days 1–9
BALB/c mice	Sc	Fragment	—	Days 1–10
Non-inbred albino Sprague-Dawley rats	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	Days 1–7
C3H/He mice	Sc	Fragment	—	Days 10–19
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–10

Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
NA	NA	NA	Weight	NA
Days 1–10	16	30	Mean ST	11–15 days
Days 1–7	16	16	Weight	1–5 g
Days 1–9	5	30	Median ST	8–11 days
Days 1–10	5	30	Median ST	9–14 days
Days 1–5	5	30	Mean ST	10–14 days
Days 1–7	8	8	Weight	2.0–4.0 g
Days 1–10	5	30	Median ST	9–13 days
Day 1 to death	5	30	Mean ST	7–11 days
Days 1–7	14	14	Weight	1.5–3.5 g
Days 1–15	5	30	Mean ST	NA
Days 1–9	5	10	Weight	5–20 g
Days 1–10	14	14	Weight	1–3 g
Days 1–7	8	8	Weight	5–8 g
Days 1–7	12	12	Weight	NA
Days 1–7	8	8	Weight	NA
Days 1–7	—	26	Weight	NA
Days 10–19	20	20	Weight	0.5–2.0 g
Days 1–10	5	30	Median ST	10–14 days

Positive control compound			One of the screening laboratories which utilized this system † (code)
NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
NA	NA	NA	Southern (08)
26271	45	≥ 135	ADL (06)
740 26271	10 10	≤ 42	Southern (08)
740	1	≥ 135	ADL (06)
740	1	≥ 135	ADL (06) Southern (08)
755	25	≥ 135	Battelle (3)
26271	50	≤ 42	Southern (08)
740	1	≥ 135	ADL (06)
NA	NA	≥ 135	MAI (01)
26271 9706	39 0.8	≤ 42	Southern (08)
749	0.75	≥ 135	Southern (08)
740	0.35	≤ 42	Southern (08)
NA	NA	≤ 42	MAI (01)
26271	19	≤ 42	Southern (08)
NS	NA	NA	Southern (08)
26271	60	≤ 42	Southern (08)
746	800	≤ 42	Southern (08)
3138	1000	≤ 42	Hazleton (02)
740	1	≥ 135	ADL (06)

Test system code[a]	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
7PM12	Plasmacytoma No. 1/triethylenemelamine (NSC-9706)	Syrian hamsters	NA	NA
7PN12	Adenocarcinoma, pancreas (No. 1)	Syrian hamsters	NA	NA
7PR12	Adenocarcinoma, prostate	Syrian hamsters	NA	NA
3PS31	P388 lymphocytic leukemia (*see* Protocol 1)	DBA/2 mice	6–7	6–7
7PT12	Carcinoma, pituitary	Syrian hamsters	NA	
3PV31	P388/vincristine (NSC-67574)	BDF, mice	6–7	6–7
3PW31	P388/NSC-38280 (a terephthalanilide)	CDBA mice	6–7	6–7
7PX12	Plasmacytoma No. 1/cyclophosphamide (NSC-26271)	Syrian hamsters	NA	NA
7P112	Plasmacytoma No. 1	Syrian hamsters	16	16
7P212	Plasmacytoma No. 2B	Syrian hamsters	NA	NA
3P421	P1534 leukemia	DBA/2 or CDBA mice	10	10–11
3P841	P815 mast cell leukemia (ascitic)	CDBA mice	7	7
3P931	P329 reticulum cell sarcoma	CDBA mice	8	8
7RC12	Adenocarcinoma, kidney	Syrian hamsters	8	8
3RO12	Ridgway osteogenic sarcoma	AKR mice	NA	NA
3RS31	Reticulum cell sarcoma (Kelley) (mouse)	CDBA mice	NA	NA
7RS12	Reticulum cell lymphosarcoma No. 5 (hamster)	Syrian hamsters	NA	NA

Testing strain	Testing (tumor) inoculum			Duration administ (non-en were al ip except a
	Site of implantation	Tissue	Level	
Syrian hamsters	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	Days 1–7
BDF$_1$ or CDF$_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1–9
Syrian hamsters	Sc	Fragment	—	Days 1–7
BDF$_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
BDF$_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1–9
Syrian hamsters	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	Days 1–7
Syrian hamsters	Sc	Fragment	—	NA
DBA/2 or CDBA mice	Ip	Spleen brei	1:100	Days 1–10
BDF$_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1–15
CDBA or BDF$_1$ mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
Syrian hamsters	Sc	Fragment	—	Days 1–7
AKR mice	Sc	Fragment	—	Days 1–7
CDF$_1$ mice	Ip	Spleen brei	1:6	Days 5–15
Syrian hamsters	Sc	Fragment	—	Days 1–7

Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
Days 1–7	12	12	Weight	NA
Days 1–7	8	8	Weight	NA
Days 1–7	8	8	Weight	NA
Days 1–9	5	30	Median ST	9–14 days
Days 1–7	12	12	Weight	1.0–2.0 g
Days 1–10	5	30	Median ST	13–17 days
Days 1–9	5	30	Median ST	9–14 days
Days 1–7	12	12	Weight	NA
Days 1–7	12	12	Weight	3.0–6.0 g
NA	NA	NA	Weight	NA
Days 1–10	5	30	Mean ST	9–11 days
Days 1–15	5	30	ST	10–14 days
Days 1–10	5	30	Median ST	13–16 days
Days 1–7	8	8	Weight	1.5–5.0 g
Days 1–7	14	14	Weight	NA
Days 5–15	NS	40	Median ST	15–20 days
Days 1–7	8	8	Weight	1.5–4.0 g

ceptable edian or n control result	Positive control compound			One of the screening laboratories which utilized this system † (code)
	NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
	NS	NA	NA	Southern (08)
	742	2.8	≤ 42	Southern (08)
	740	8	≤ 42	Southern (08)
days	27640	10	≥ 135	
	19893	20		
	60339	16		
	26271	22		
2.0 g	26271	56	30	Southern (08)
7 days	26271	22	≥ 135	ADL (06)
days	26271	22	≥ 135	
	NS	NS	≤ 42	Southern (08)
5.0 g	26271	10	≤ 42	Southern (08)
	NA	NA	≤ 42	Southern (08)
days	49842	2.5	≥ 135	
4 days	740	1	≥ 135	Southern (08)
6 days	749	100	≥ 135	
	740	1		
5.0 g	740	12	≤ 42	Southern (08)
	NA	NA	NA	ADL (06)
0 days	26271	45	≥ 135	ADL (06)
1.0 g	740	12	≤ 42	Southern (08)

Test system code[a]	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
3SA12	Sarcoma 180	Swiss mice	7	6–7
7SB12	Adenocarcinoma, small bowel	Syrian hamsters	10–12	10–12
5TG42	Dunning leukemia/thioguanine riboside (NSC-29422)	Fischer rats	10–12	10–12
5WA12	Walker carcinosarcoma 256	Non-inbred albino Sprague-Dawley or Fischer rats	11–13	11–13
5WA16	Walker carcinosarcoma 256 (*see* Protocol 1)	Non-inbred albino Sprague-Dawley or Fischer rats	10–12	10–12
5WA31	Walker carcinosarcoma 256	Non-inbred albino Sprague-Dawley or Fischer rats	11–13	11–13
5WA39	Walker carcinosarcoma 256	Non-inbred albino Sprague-Dawley rats	7–9	NA
5WC12	Walker carcinosarcoma 256/cyclophosphamide (NSC-26271) (subcutaneous)	Non-inbred albino Sprague-Dawley rats	11–13	11–13
32X31	P288/methotrexate (NSC-740)	DBA mice	5–7	5–7
32512	Carcinoma 1025	CHKRF$_1$ or AKR mice	14	12–14
32831	P288 lymphocytic leukemia	DBA mice	5–7	5–7
34A42	L4946/azaserine (NSC-742)	AKR mice	NA	NA

Testing strain	Testing (tumor) inoculum: Site of implantation	Tissue	Level	D
Swiss mice	Sc	Fragment	—	Days 1
Syrian hamsters	Sc	Fragment	—	Days 1
Fischer rats	Sc	NS	—	Days 1
Non-inbred albino Sprague-Dawley or Fischer rats	Sc	Fragment	—	Days 1
Non-inbred albino Sprague-Dawley or Fischer rats	Im	Ascitic fluid Brei	10^6 cells 1:6	Days 3
Non-inbred albino Sprague-Dawley or Fischer rats	Ip	Ascitic fluid	10^6 cells	Days 1
Non-inbred albino Sprague-Dawley rats	Iv Iv	Ascitic fluid Brei	10^6 cells 1:50	Days 1
Non-inbred albino Sprague-Dawley rats	Sc	Fragment	—	Days 1
BDF_1 or DBA/2 mice	Ip	Ascitic fluid	10^6 cells	NA
$CHKRF_1$ or AKR mice	Sc	Fragment	—	Days 1
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–1
$CHKRF_1$ mice	NS	NS	NS	Days 1–1

Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
Days 1–7	8	8	Weight	0.5–2.0 g
Days 1–11	12	12	Weight	0.8–3.5 g
Days 1–12	5	30	ST	11–13 days
Days 1–5	Day of evaluation	10–14	Weight	3–22 g
Days 3–6	7	7	Weight	3–12 g
Days 1–9	5	30	Median ST	5–9 days
Days 1–5 or 5–10	NA	30	ST	10–15 days
Days 1–5	Day of evaluation	10–14	Weight	3–12 g
NA	5	30	Median ST	9–12 days
Days 1–5	15	15	Weight	0.5–2.0 g
Days 1–10	5	30	Median ST	7–9 days
Days 1–11	NA	NA	Mean ST	NA

Acceptable median or mean control result	Positive control compound			One of the screening laboratories which utilized this system † (code)
	NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
2.0 g	7365	0.02	≤ 42	
	3051	200		
	26271	45		
3.5 g	26271	50	≤ 42	Southern (08)
13 days	NA	NA	≥ 135	
2 g	9706	0.05	≤ 42	
2 g	45383	0.2	≤ 42	
	26271	2.5		
days	26271	2.5	≥ 135	
	19893	100		
15 days	26271	10–20	≥ 135	ADL (06)
2 g	NS	NS	≤ 42	WARF (05)
2 days	NA	NA	≥ 135	ADL (06)
2.0 g	3052	50	≤ 42	Battelle (03)
days	740	1	≥ 135	ADL (06)
	740	1.5	≥ 135	Southern (08)

Test system code[a]	Tumor	Propagation strain and species	Transfer day for propagation	Transfer day for testing
34942	L4946 lymphatic leukemia (solid)	AKR mice	NA	NA
35P21	P335 leukemia	CDBA mice	7	7
36T21	L1210/6-thioguanine (NSC-752)	NA	NA	NA
37P21	Ca755/6-mercaptopurine (NSC-755) (solid)	C57BL/6 mice	NA	NA
38C12	P1798/cortisone (NSC-9703)	CAF_1 mice	14	12–14
38P12	P1798 lymphosarcoma	CAF_1 mice	14	12–14
38121	P1081 chloroleukemia	BDF_1 mice	NA	NA
39112	S91 Cloudman melanoma	BDF_1 or DBA/2 mice	21	21
39831	C1498 myeloid leukemia	C57BL/6 mice	9–10	9–10

Testing strain	Testing (tumor) inoculum			Duration admin (non-c were i except
	Site of implantation	Tissue	Level	
$CHKRF_1$ mice	NS	NS	NS	Days 1–11
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–9
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–9
BDF_1 mice	Sc	Fragment	—	Days 1–11
CAF_1 mice	Sc	Fragment	—	Days 1–11
BALB/c or CDBA or CAF_1 mice	Sc	Fragment	—	Days 1–11
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–10
BDF_1 mice	Sc	Fragment or brei	1:2	Days 1–11
BDF_1 mice	Ip	Ascitic fluid	10^6 cells	Days 1–10

Duration of drug administration (non-endocrines were all given ip qd except as noted)	Day of initial recording of survivors on screening data summary for acute toxicity	Day of evaluation	Parameter	Acceptable median or mean control result
Days 1–11	NA	NA	ST	10–13 days
Days 1–9	5	30	Mean ST	7–10 days
Days 1–9	5	30	ST	8–11 days
Days 1–11	12	12	Weight	0.5–2.0 g
Days 1–11	12	12	Weight	0.5–2.0 g
Days 1–11	12	12	Weight	0.5–2.0 g
Days 1–10	5	30	ST	8–12 days
Days 1–11	21	21	Weight	0.5–2.0 g
Days 1–10	5	30	ST	8–12 days

able or ntrol lt	Positive control compound			One of the screening laboratories which utilized this system † (code)
	NSC No.	Dose (mg/kg/injection)	Acceptable T/C%	
ys	740	1	≥ 135	Southern (08)
s	740	1.5	≥ 135	MAI (01)
s	740	1.5	≥ 135	Southern (08)
	NS	NS	≤ 42	Southern (08)
	19893	159	≤ 42	MAI (01) ADL (06) Southern (08)
	9703	25	≤ 42	MAI (01) ADL (06) Southern (08)
s	740	1	≥ 135	ADL (06) MAI (01)
	26271	25	≤ 42	MAI (01)
s	740	1	≥ 135	ADL (06)

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